

Appendix Z Seabed monitoring report



Ngāi Tahu Seafood Resources Limited

Hananui Aquaculture Project

Seabed monitoring

Evidence of Emily McGrath regarding *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* and
Proposed Conditions

Emily McGrath
11-13-2025

Introduction

My name is Emily McGrath.

My role in relation to the Hananui Aquaculture Project (“**HAP**”) has been to provide expert evidence in relation to seabed monitoring. I was the lead author of the *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* which is provided within **Appendix Z** of the application.

This evidence has been prepared to accompany the application by Ngāi Tahu Seafood Resources Limited (“**NTS**”) for approvals required for the HAP under the Fast-track Approvals Act 2024 (“**FTAA**”). It has been prepared on the understanding that the process for determining applications under the FTAA does not require a hearing to be held, and accordingly the purpose of this evidence is to confirm that, relative to my area of expertise, the *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* provides an appropriate description of the relevant environment, the proposed activities comprising the effects of the HAP on that environment, and the way those effects are proposed to be managed.

My findings are set out in full in the *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* included within **Appendix Z** of the application.

While this application is not being considered by the Environment Court, I confirm that I have read the Code of Conduct for expert witnesses contained in the Environment Court of New Zealand Practice Note 2023 and that I have complied with it when preparing this evidence. Other than when I state I am relying on the advice of another person, this evidence is within my area of expertise. I have not omitted to consider material facts known to me that might alter or detract from the opinions that I express.

Qualifications and Experience

I am a Marine Ecologist.

I graduated from Victoria University of Wellington with a PhD in Marine Science.

I am a marine ecologist with over 10 years of experience evaluating the ecological effects of aquaculture and other human activities on Aotearoa New Zealand’s coastal and benthic environments. My work at the Cawthron Institute focuses on seabed monitoring, habitat assessment, and site suitability evaluations that support sustainable aquaculture development, including several open-ocean salmon farming initiatives. I have led and managed large commercial and applied research projects nationwide, encompassing environmental impact assessments, routine marine monitoring, and investigations into benthic species’ physiological responses to aquaculture stressors. This work has contributed to the development of environmental health metrics and strengthened New Zealand’s capability in offshore aquaculture environmental management.

I have authored or co-authored 40+ technical reports relating to aquaculture impact, environmental monitoring and effects assessments, ecological site-scoping for future developments and management planning.

In proving this evidence in relation to seabed monitoring, I have considered the following matters as relevant to that topic:

- The project description provided by NTS as set out in section 6 of the application;
- The description of the existing environment, the effects of the HAP on that environment and their significance, and the proposed management and mitigation measures to manage those effects all as set out in the assessment of environmental effects accompanying the application;
- The technical assessments of
 - *Assessment of seabed effects associated with farming salmon offshore of northern Stewart Island / Rakiura*, Bennett et al. 2022, 2025;
 - *Seabed monitoring at the proposed Hananui salmon farming area: approach to zoning, environmental standards, and monitoring for Stage 1*, Newcombe et al. 2022;
 - *Best practice guidelines for benthic and water quality monitoring of open ocean finfish culture in New Zealand*, Giles et al. 2021;
 - *Best management practice guidelines for salmon farms in the Marlborough Sounds: Part 1: Benthic environmental quality standards and monitoring protocol (Version 1.2 August 2022)*, Fletcher et al. 2022;
 - *Recommendations for a Seabed Management Plan for 'Blue Endeavour' salmon farming area. Prepared for The New Zealand King Salmon Co. Limited*, Elvines et al. 2021; and
- A range of references describing the existing seabed environment including sediment and habitat types, as well as characteristics of the biogenic habitat observed during habitat mapping exercises
- MPI site scoping investigations for the area

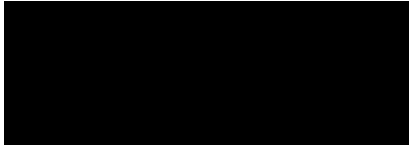
Confirmation of Contents of Report and Proposed Conditions

I confirm that in my opinion the *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* contain an accurate and appropriate description of the environment, the actual and potential effects of the HAP, and the recommended actions to manage those effects within my area of expertise.

I confirm that in my opinion the contents of the *Recommendations for seabed monitoring at the proposed Hananui salmon farming area* may be relied on in making a decision on the approvals sought for the HAP and confirm that provided effects within my area of expertise are managed as

proposed in the application those effects will not be unacceptable and will be managed to a standard that I consider meets good practice.

I confirm that I have reviewed the conditions that NTS proposes for the approvals being sought as they relate to my area of expertise. I confirm that in my opinion, those proposed conditions are appropriate.



Emily McGrath



Recommendations for seabed monitoring at the proposed Hananui salmon farming area

Cawthron Report 4195

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Recommendations for seabed monitoring at the proposed Hananui salmon farming area

Emily McGrath, Holly Bennett

Prepared for Ngāi Tahu Seafood Resources



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1. Introduction

1.1 Background

Ngāi Tahu Seafood Resources (Ngāi Tahu Seafood) are applying to develop the Hananui Aquaculture Project, a two-stage salmon farming development within an approximately 1,285 ha area (the Hananui proposal area; Figure 1) located 2–6 km offshore of Rakiura / Stewart Island (hereafter Rakiura).

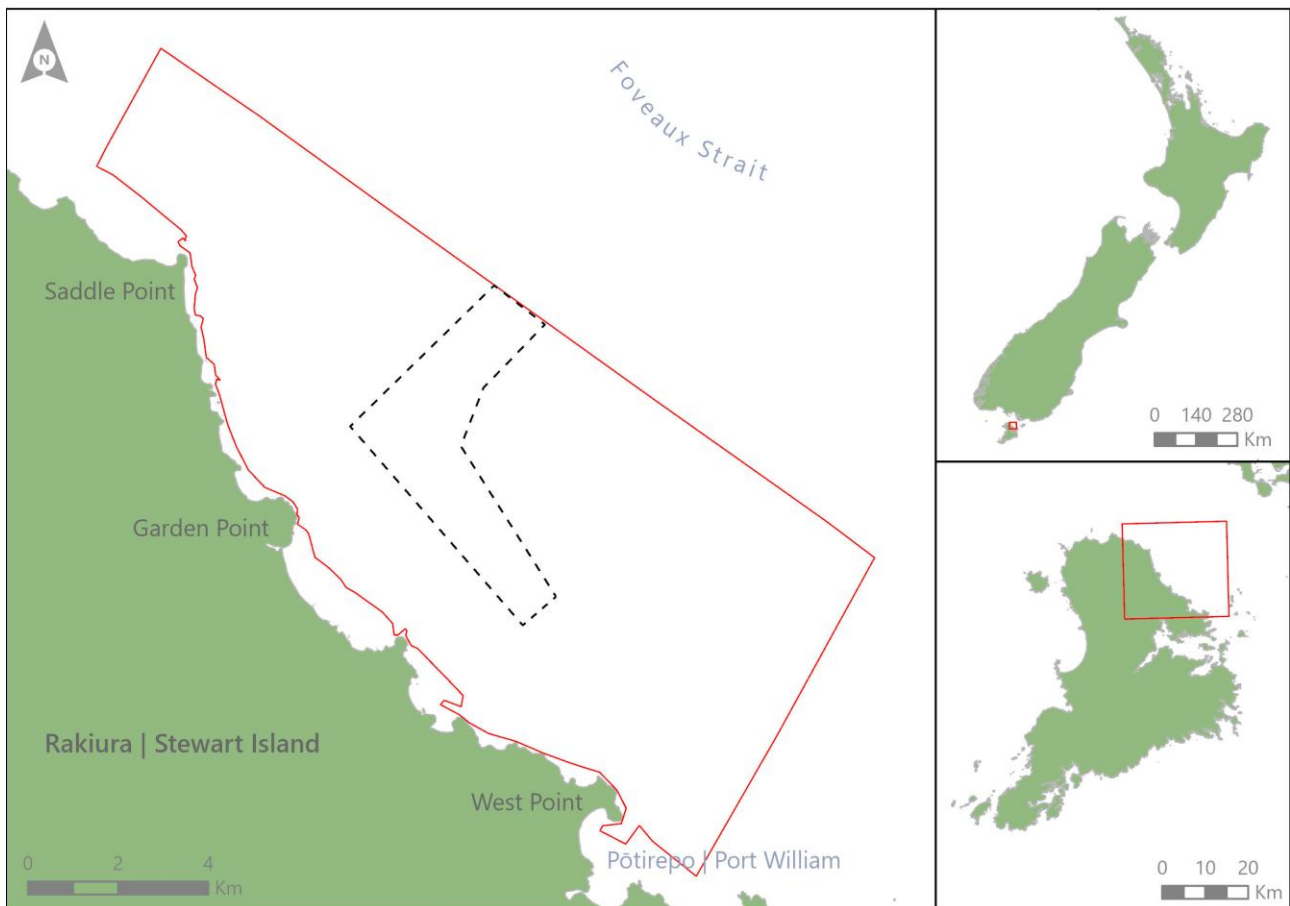


Figure 1. The Ngāi Tahu Seafood Hananui 'proposal area' (black dashed line) and the broader 'survey area' (red polygon, left panel), which encompasses the area where data were collected from: Ngāi Tahu Seafood contracts (2018–25); information from preliminary scoping investigations commissioned by the Ministry for Primary Industries (MPI) in 2018; and a seabed mapping exercise survey conducted as part of a wider Land Information New Zealand (LINZ) survey in early 2022.

The development will consist of up to four farms, each divided into two blocks (A and B) with 10 circular fish pens. Development will occur in stages: during Stage 1 only a single block per farm unit will be operational, with up to 15,000 tonnes of feed discharged per year in total. Ngāi Tahu Seafood propose

to farm under a single year class regime, whereby feed discharges will vary substantially throughout the production cycle of each farm block. At the beginning of Stage 1, fish will be stocked at 6-month intervals for each of the farm blocks, with peak production staggered across the four farms. Feed input will increase leading up to harvest, then rapidly decrease to zero during the harvesting period, and remain at zero before fallowing. Each site will be fallowed for at least 3 months between production cycles.

Habitat mapping of the wider survey area identified six primary seabed habitat types (Bennett et al. 2025). For monitoring, these have been consolidated into two overarching categories: sandy and biogenic. Sandy habitats comprise sand, sandy shell hash and coarse gravel–shell–sand substrates, whereas biogenic habitats comprise bushy-bryozoan thickets, bryozoan–sponge reefs, and patchy low-relief bryozoan–sponge habitats (Figure 2).

Farm locations have been selected to minimise interactions with ecologically sensitive biogenic habitats, and the predicted primary organic deposition is constrained to sandy seabed habitats. While measurable change may occur in these sandy habitats, significant effects¹ are unlikely at Stage 1. Low-level resuspension will disperse material beyond the farm location, possibly reaching into more distant biogenic habitats. These far-field accumulations are expected to be low, with only minor effects that will likely be difficult to detect.

To ensure ecological risks (e.g. undesirable or unexpected effects) are managed appropriately, monitoring will be required. This monitoring will validate depositional model predictions and inform adaptive management of seabed effects, supporting decision-making for progression between stages. A zone-based approach is proposed (Section 2), designed for the highly dispersive Hānau environment. The monitoring approach is specifically tailored to each habitat (sandy and biogenic) for the Stage 1 development. As this is a new farming area with habitats unlike those farmed elsewhere in Aotearoa New Zealand, uncertainties remain around their response to organic enrichment. A precautionary monitoring approach, refined as new data become available, is proposed before progression to Stage 2 is permitted.

1.2 Scope

This report provides preliminary recommendations on seabed monitoring approaches, indicators and threshold frameworks to support the current Fast-track Approvals application (FTAA). It draws on and complements the seabed effects assessment (Bennett et al. 2025), which details the staging approach, depositional modelling, and the anticipated scale and nature of environmental change due to farm activities.²

This report outlines the seabed monitoring framework for Stage 1 of the two-stage development and provides preliminary recommendations to inform the baseline monitoring plan, the environmental monitoring plan (EMOP) and the environmental management plan (EMAP). Monitoring requirements

¹ Sufficient to cause ecological degradation or loss of biodiversity.

² See Bennett et al. (2025) for additional background information.

under the consent are expected to evolve as understanding of the environment and effects of the marine farm improve and as best practice monitoring methods advance. The zone boundaries predicted depositional footprints and monitoring stations presented here are based on Stage 1 modelling. The Stage 1 monitoring framework has been designed so that core stations are retained for Stage 2 monitoring, with reference stations held constant across all stages to provide a stable basis for evaluating environmental change throughout the full farm-development phase. While many core aspects (e.g. reference station location, sampling approaches) will remain applicable to later phases, the spatial configuration of Zones 1, 2 and Beyond Zone 2 will be revised as part of the progression to Stage 2 (outlined in Section 7).

The objective of this report is to demonstrate how monitoring can be structured to detect, validate and manage these effects in practice, drawing on guidance from the open ocean aquaculture best management practice guidelines (Giles et al. 2021). This current document does not constitute a draft EMOP. The EMOP will be prepared at a later stage in accordance with consent conditions and will be certified by Environment Southland following refinement through stakeholder feedback, submissions and any future adjustments to the farming plan.

2. Zones approach to management

Salmon farm monitoring in Aotearoa New Zealand commonly applies a zone-based, tiered monitoring approach for assessing seabed effects from marine farms. This framework recognises that depositional effects are most pronounced in proximity to the farm and decrease with distance. Consistent with this approach, it is proposed that the seabed effects associated with the waste discharge from the Hananui proposal area should be managed under a zones approach. This approach should comprise three management zones that traverse the area of expected maximum effect to beyond where potential effects are expected to diminish.

Monitoring zones for Stage 1 (Figure 2) are defined using Stage 1 depositional modelling, as detailed in the seabed effects assessment (Bennett et al. 2025).

- **Zone 1** (blue line in Figure 2) encompasses the **zone of maximum effect** corresponding to the $0.5 \text{ kg}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ solids flux contour,³ which represents the boundary for predicted effects with no resuspension processes considered. Within this footprint, it is predicted that between 10 ha and 19 ha of sandy seabed (0.8% to 1.5% of the proposal area) may receive a high level of deposition of organic waste per farm block during peak feed input at Stage 1.
- **Zone 2** (orange line in Figure 2) encompasses the **primary footprint** ($7 \text{ g}\cdot\text{m}^{-2}$ residual solids⁴ contour). This zone comprises between 473 ha and 533 ha of sandy seabed (37% to 42% of the proposal area) outside of Zone 1, which may receive a moderate level of deposition per farm block at Stage 1. This footprint considers the influence of resuspension processes on waste dispersal dynamics and generally aligns with the proposal area boundary, although it extends beyond the boundary in places where the residual solids contour crosses the proposal area boundary.
- **Beyond Zone 2** is a spatially defined area extending 3 km in the predominant current flow direction (northwest / southeast) and 2 km cross-current from the Zone 2 boundary. This area encompasses the $0.7 \text{ g}\cdot\text{m}^{-2}$ residual solids footprint (or outer limit of effects [OLE]; Bennett et al. 2025) and extends beyond it to capture the maximum spatial extent at which farm-related effects may be detectable.

The areas outside of the Beyond Zone 2 area exhibit natural conditions with no effect expected from farm activities.

The zones are defined such that monitoring effort can be appropriately focused to ensure that the worst affected areas do not exceed the maximum permitted level of enrichment, and that effects do not extend beyond the predicted effects area.

³ Solids flux refers to the rate at which particulate matter is deposited onto the seabed surface from the overlying water column in the absence of resuspension (Bennett et al. 2025).

⁴ Residual solids refer to the particulate waste material that remains on the seabed, accounting for secondary transport through resuspension (Bennett et al. 2025).

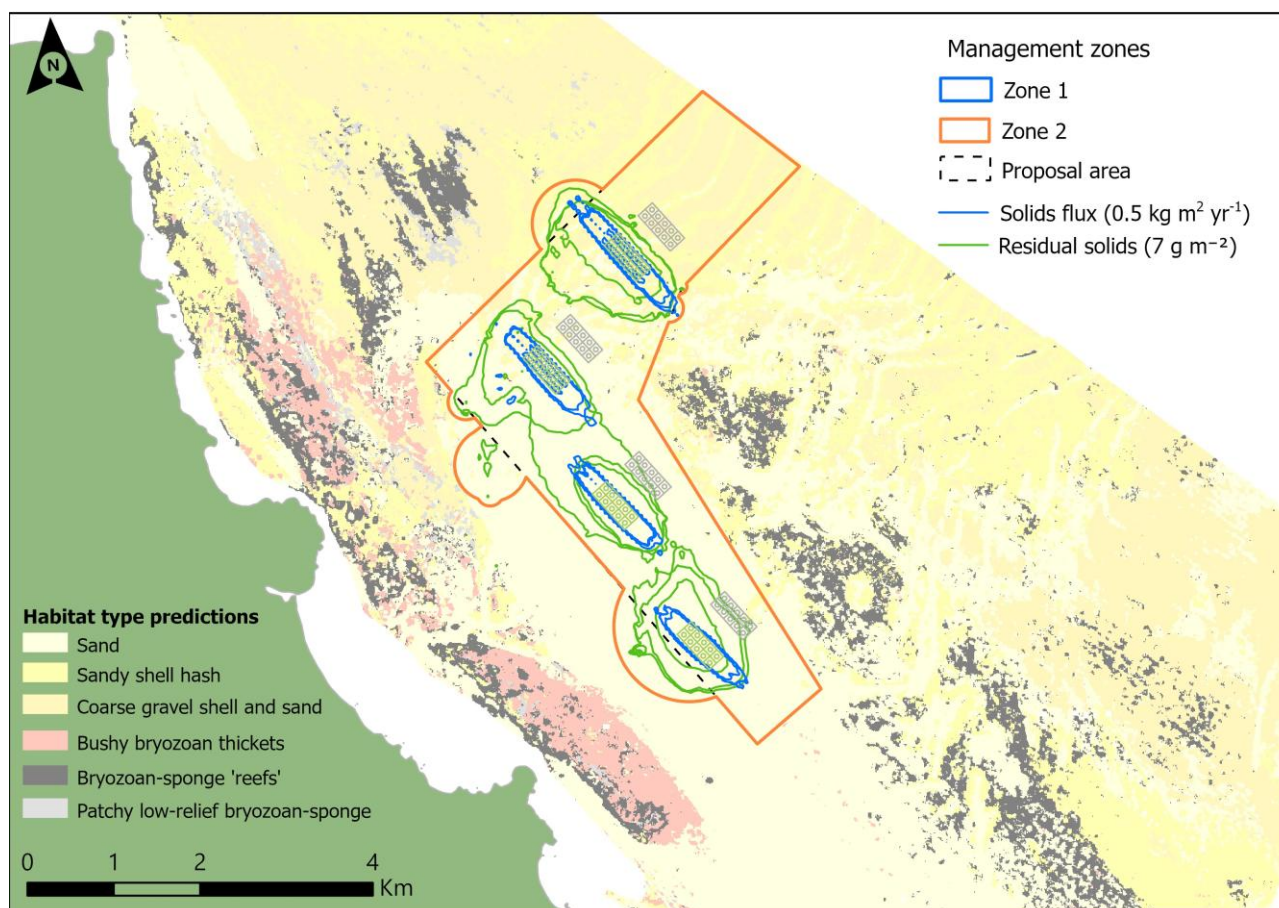


Figure 2. Hananui proposal area with overlaid management zones based on Stage 1 feed inputs. Zone 1 = the zone of maximum effect defined by the $0.5 \text{ kg} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ solids flux footprint, Zone 2 = the primary footprint defined by the $7 \text{ g} \cdot \text{m}^{-2}$ residual solids footprint, Beyond Zone 2 = spatially defined area 3 km northwest / southeast and 2 km cross-current from the Zone 2 boundary.

3. Baseline data collection

Baseline surveys are designed to capture the environmental state prior to any farm influence and establish reference conditions against which potential farming effects at both Stage 1 and Stage 2 can be distinguished. These surveys must characterise both spatial and temporal variability within the Hananui site to enable robust future comparisons. Baseline monitoring locations should be guided by existing information, including the habitat maps that were derived using visual survey methods and predictive modelling based on acoustic seabed data (Bennett et al. 2025).

Because the zoning framework and station layout presented in this report apply specifically to Stage 1 of the two-stage development, sampling locations established during baseline will be retained for Stage 1 monitoring to maintain spatial consistency and allow meaningful temporal analysis of benthic condition.

Given the dynamic conditions of Foveaux Strait, multi-seasonal sampling, including sampling timed around extreme weather events, is essential to define the natural range of environmental variation and to distinguish farm-related change from background variability. Areas not previously sampled during earlier investigations should be included to ensure comprehensive baseline coverage across the proposal area. Where possible, historical data will be incorporated into the baseline monitoring design (e.g. Bennett et al. 2025).

This section outlines the key considerations for baseline data collection across the habitat types present at the Hananui site. Detailed monitoring requirements are presented separately for sandy (Section 4.2) and biogenic habitats (Section 5) to accommodate the different habitat structure and sensitivity to disturbance.

Baseline monitoring considerations include:

- **Duration and timing:** Sampling must be undertaken across multiple seasons over a minimum 2-year period for biogenic habitat and 1 year for sandy habitat⁵ to capture natural interannual⁶ and seasonal environmental and ecological variability. If possible, additional surveys should target the period after major weather events (e.g. prolonged high winds or swell) to assess their effect on biogenic habitats and clarify the natural movement and redistribution of sandy substrates. Baseline sampling must be completed prior to fish stocking but may be ongoing during the installation of farm structures.
- **Spatial and ecological characterisation:** For sandy habitats, baseline sampling must capture the range of sediment types and associated macrofaunal communities across the site to enable meaningful temporal and spatial comparisons between habitat substrata as well as any areas that have not been previously sampled. For biogenic habitats, large-scale mapping to confirm their spatial extent (i.e. to map changes since the previous multibeam survey in 2022; Bennett et al.

⁵ However, this period may be extended where high spatial or temporal variability is observed, as determined through expert judgement.

⁶ It is acknowledged that a single year of data will not capture interannual variability in the case of sandy habitats.

2025), while video surveys will characterise community assemblages and their condition and inform the placement of suitable reference locations.

- **Sampling design:** The baseline design should align conceptually with the proposed routine monitoring framework, using consistent sampling locations and indicators, and replication to support appropriate statistical power for the required effect detectability in ongoing monitoring and 'before–after' comparisons.
- **Refinement of routine monitoring:** Baseline results will inform refinement of future monitoring parameters, including the selection of sampling locations, replication levels and methodologies.
- **Documentation:** The baseline survey design and implementation must be set out in a certified, standalone Baseline EMOP prior to commencement of routine monitoring.

4. Sandy seabed habitats

In this section, we outline the proposed parameters and sampling design for monitoring sandy seabed environments.

4.1 Sample type / parameters

A range of parameters, shown in Table 1, may be measured to assess the intensity of seabed enrichment across the site. Three main sample types should be used:

- **Sediments:** collected with a grab sampler, from which a range of parameters could be measured either with sensors employed *in situ*, or with subsequent laboratory processing.
- **Water:** *in situ* measurement of water column dissolved oxygen.
- **Visual:** seabed imagery collected using a ROV or video sled.

Some measurements will yield immediate results; others require laboratory processing and thus additional turnaround time (Table 1). Note that with regard to the macrofaunal community indices referred to in Table 1, infaunal samples at some stations may yield only abundance and taxonomic diversity; this is because the infauna in some areas are naturally too depauperate to reliably use more complex calculated community metrics (see Bennett et al. 2025). The use of larger infaunal samples (either full grabs, or a composite of replicate cores) may enable reliable calculation of additional macrofaunal community metrics; however, this substantially increases collection and processing times as well as cost.

Some indicators may, in future, be replaced by alternative metrics once new methods have been validated for monitoring and shown to reliably correspond with infaunal community responses (Giles et al. 2021). One promising approach is the use of bacterial environmental DNA (eDNA) to assess benthic functioning and enrichment state. The eDNA-derived bacterial Metabarcoding Biotic Index (b-MBI) can substitute for the macrofaunal component in calculating enrichment stage (ES), offering a non-invasive and scalable alternative for future monitoring. This is currently used as a reliable indicator of farm-derived organic enrichment in the Marlborough Sounds (Fletcher et al. 2022) and shows promise at the Hananui proposal area (Appendix 1).

Table 1. Recommended broad parameters and their role in monitoring of sandy seabed environments at the proposed Hananui salmon farming area. EQS = environmental quality standards, against which acceptability of farming impacts can be assessed. Initial EQS are proposed in Table 4. The applicability and sensitivity of individual parameters under local environmental conditions will be evaluated and confirmed during baseline monitoring.

Parameter	Sample type	Possible measurement method	Approximate time frame for results	Purpose / use
Sulphides	Benthic grab	Laboratory processing*	Immediate (method-dependent)	Initial EQS
Redox / pH		Electronic probe <i>in situ</i>	immediate	Initial EQS
Infaunal community metrics		Laboratory processing – calculation of metrics (e.g. abundance, diversity)	6–8 weeks +**	Initial EQS
Bacterial DNA / other eDNA			4 weeks +	Initial EQS Model validation***
Grain size		Laboratory processing	4 weeks +	Context for interpretation of other parameters
Contaminant levels, incl. zinc, trace metals (beneath-farm)			4 weeks +	Initial EQS****
Organic matter tracers			4 weeks +	Model validation and weight of evidence for causality
Near-bottom water column dissolved oxygen	Oxygen sensor	Electronic sensor attached to benthic grab	Immediate	Initial EQS
Bacterial mat / outgassing†	Seabed imagery	Visual assessment of areal coverage of bacterial mat, presence / absence of outgassing	Immediate	Initial EQS
Epifaunal community composition†	Drop-cam, ROV or similar	Assessment of epifauna and other conspicuous indicators (manual or with image analysis software)	Review immediate, quantification 4 weeks +	Initial EQS and context for interpretation of other parameters
Additional parameters proposed for model validation				
Fatty acid composition	Benthic grab	Laboratory processing		Model validation

* At least two laboratory methods are available. Ideally, ultraviolet (UV) spectrophotometry of sediment pore water would be used, but this method requires further development of method-specific thresholds; this work is ongoing. Total free sulphides have traditionally (e.g. Fletcher et al. 2022) been measured with the ion-selective electrode method. Note that appropriate standards may differ depending on which method is used. Any change between methods over time should ensure that data remain comparable.

** Processing time will increase significantly if larger infaunal samples are required.

*** For example, as a means for progressing between stages (see Section 7).

**** Sediment metal concentrations should be assessed against the widely accepted default guideline (DGV) of ANZG (2018) and will not be considered further in this document.

† We note that visual assessments of seabed enrichment (e.g. from video imagery) generally exhibit low detection sensitivity under highly dispersive conditions, particularly for distinguishing moderate to intermediate enrichment effects (Giles et al. 2021).

4.2 Survey design

The survey design aligns with the rotational farming approach, in which peak production is staggered across the four farms. At any given time, only one farm block will operate at peak production, while the others remain at lower feeding intensity or in fallow. Accordingly, the tiered monitoring framework concentrates sampling effort on the farm block at peak production to validate modelled effects and characterise the benthic response to maximum enrichment. Concurrently, lower-intensity monitoring is maintained at the remaining farms to detect early indicators of change, verify recovery during low-feed or fallow periods, and ensure environmental conditions are preserved. This approach provides an efficient, risk-proportionate monitoring regime consistent with operational staging and predicted depositional patterns, targeting areas of highest predicted effect (Giles et al. 2021).

Initial sampling locations should be selected based on information from depositional modelling outputs and the available habitat map for the site (Bennett et al. 2025). Locations may be refined following the baseline survey or any subsequent depositional footprint mapping exercise. Sampling locations established during the baseline phase should be retained for Stage 1 monitoring to ensure direct comparability between monitoring phases and enable temporal analysis of benthic response.

Monitoring effort should be proportionate to the level of certainty regarding depositional effects and benthic response (Giles et al. 2021). In the initial years of operation, when uncertainty is greatest, sampling intensity may be elevated – particularly at farm blocks operating at peak production – to increase confidence in model predictions and develop a robust understanding of benthic response to enrichment. As certainty increases and benthic response patterns become better defined, monitoring intensity may be reduced.⁷ Station placement will also be designed to maintain continuity through to Stage 2 monitoring.

A tiered monitoring strategy is recommended, whereby more cost-effective and rapid Tier 1 indicators are used routinely to assess whether results fall within determined thresholds, or if Tier 2 sampling is required. The monitoring strategy will vary by zone, with Zone 1 incorporating two approaches (full suite and tiered) based on farm stocking levels (see below). For tiered surveys, Tier 1 indicators should include total free sulphides, redox / pH index and b-MBI⁸ as well as an assessment of near-bottom water column dissolved oxygen (DO), white, anaerobic bacterial coverage and outgassing from sediments. Tier 2 indicators should include AZTI's Marine Biotic Index (AMBI) and the Benthic Quality Index (BQI; see Section 4.4 for further detail), plus a qualitative / expert judgement assessment of the other available macrofaunal indicators. Where Tier 1 trigger values are exceeded, additional sampling using the more comprehensive Tier 2 indicators can be undertaken.

Section 4.4 provides further detail on environmental quality standards (EQS) for sandy seabed habitats and tier-specific thresholds.

⁷ This process will be outlined in the EMAP and EMOP and will likely require agreement with expert opinion and Environment Southland.

⁸ Incorporation of b-MBI at the Hananui proposal area will require validation against infaunal data before it can be considered a standalone Tier 1 indicator.

Zone 1

Two monitoring strategies, reflecting different levels of sample analyses, are recommended based on the stocking level of the farm:

- **Full suite:** Tier 1 and Tier 2 indicators are collected and analysed at all stations. This strategy is important for the baseline survey to (i) assess the natural variability of all indicators across the site⁹ and (ii) to ensure Tier 1 indicators are sufficiently sensitive and correspond well with Tier 2 indicators. This full-suite sampling strategy is also recommended for farms in peak production.
- **Tiered:** Both Tier 1 and Tier 2 indicators should be collected, but only the rapid, cost-effective Tier 1 indicators will be routinely analysed. Tier 2 samples should be archived and analysed only if two or more Tier 1 indicators exceed trigger levels. This tiered sampling strategy is applied to farms that are not stocked at peak levels or are fallowed (interim stations).

At least one sampling station should be positioned at the pen edge for each farm block (or at the anticipated pen edge for baseline surveys) in areas of predicted areas of maximal effect (AME; Giles et al. 2021). Operators may also choose to sample an additional three to five stations at the pen edge to capture local-scale variability.

To characterise the extent of farm effects predicted to occur within Zone 1, two additional stations should be positioned along the Zone 1 boundary, corresponding to the edge of the predicted zone of maximum effects (ZME) footprint. Stations should be placed along the prevailing current axis (northwest–southeast) to ensure detection of footprint elongation due to current directionality and coverage within the boundary of specified effect level¹⁰ (BSEL; Giles et al. 2021).

To capture areas of possible accumulation¹¹ (APA; Giles et al. 2021) from neighbouring farms (even if they are not at peak production), one Zone 1 monitoring station (interim APA station; Figure 3, indicated in orange) should be located adjacent to each remaining farm.

Stage 1 monitoring scenarios will be detailed in the EMOP, but Figure 3 provides an example scenario of sampling against farm block stocking cycles similar to what would happen in Stage 1. In this scenario, farms at peak production (indicated by single red star) are monitored using the full-suite monitoring approach, and farms not at peak stocking or those that are fallowed (indicated by double red stars) use a tiered approach.

⁹ If variability is low, only a sub-set of these samples may need to be processed.

¹⁰ Defined as the allowable degree of change from background organic enrichment conditions (Giles et al. 2021).

¹¹ Depositional footprints from individual blocks are overlapping; therefore, effects are not independent to each farm; effects within each block will thus be influenced by neighbouring blocks, including residual deposition from those stocked earlier in the cycle.

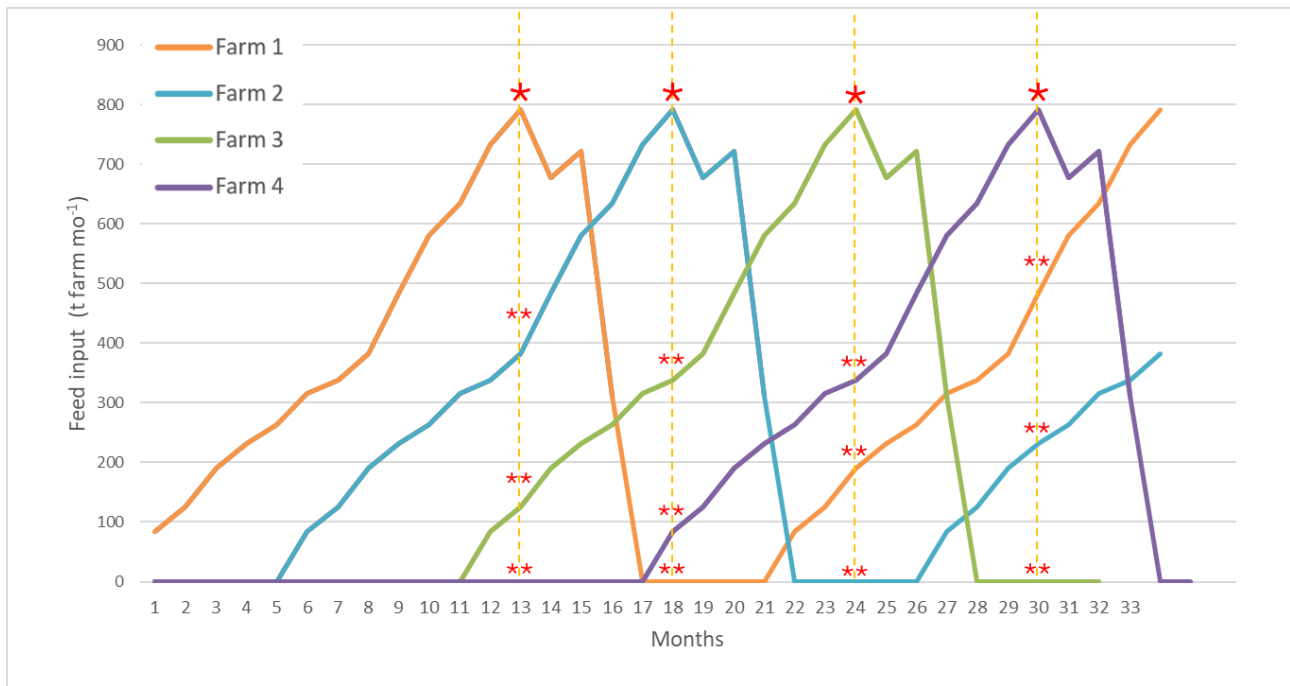


Figure 3. Representation of monitoring approach for Zone 1. The red asterisks (*) indicate the farm block at peak production that will undergo full-suite monitoring; the remaining stocked farms not at peak production (interim stations) will undergo a tiered monitoring approach (indicated by double red asterisks [**]).

Zone 2

The tiered monitoring strategy should be used for all Zone 2 stations (Figure 4, indicated in purple), whereby only Tier 1 indicators would be routinely collected and analysed. Monitoring stations will be distributed across the zone polygon to capture both predicted effects and their spatial extent, including areas further from the farm block (Figure 4). Stations should be placed so that 50% are within the predicted primary footprint (e.g. within the residual solids contour) of the nearest farm to capture areas of possible accumulation, and along the edge of the Zone 2 boundary to capture predicted farm effects, particularly in the direction of the prevailing current (northwest / southeast). It may also be necessary to stratify (and classify) these areas according to sediment grain size and macrofaunal abundance and diversity, such that they are comparable to appropriate reference sites for statistical comparisons.

Beyond Zone 2

A tiered monitoring strategy should be applied to all Beyond Zone 2 monitoring stations (Figure 4, indicated in blue). As with Zone 2, only Tier 1 indicators would be routinely collected and analysed.

Monitoring stations should be located at the edge of the OLE ($0.7 \text{ g} \cdot \text{m}^{-2}$ residual solids footprint) boundary to capture the extent of predicted effects, as well as at the outer boundary of Beyond Zone 2, which will inform the maximum spatial extent where farm-related effects may be detectable (Figure 4). Beyond Zone 2 sampling stations may also be stratified similarly to the Zone 2 stations (e.g. according to sediment grain size and macrofaunal characteristics).

Reference stations

The environment outside of the farmed area is expected to be quite heterogeneous, so a relatively high number of reference sampling stations will be required to ensure that adequate statistical power for detecting farm-related effects is achieved. It will also provide the consent holder with clear benchmarks against which potential effects can be reliably assessed throughout the duration of farming operations. Replicate and station number requirements for video surveys should be determined through power analysis of baseline data to ensure appropriate replication for effect detection (Giles et al. 2021).

Reference stations should be stratified using the same criteria applied to Zone 2 stations to ensure robust and comparable assessments. At least 10 stations should be established during baseline monitoring, with a tiered approach applied to reference stations where only Tier 1 indicators will be routinely collected and analysed. Stations should be positioned at a distance of at least twice that of the predicted effects footprint in the relevant direction from the farm, accounting for Stage 2 development (Giles et al. 2021).

Example survey design scenario

Figure 4 illustrates a seabed monitoring scenario where Farm 1A is at peak production:

- Three **Zone 1 AMA stations** (red) are positioned around the block in peak production, with two additional stations located on the Zone 1 boundary (BSEL) along the main current axis (northeast–southwest).
- One **interim APA station** (orange) is positioned adjacent to each remaining farm block to assess potential cumulative effects.
- **Zone 2 stations** (purple) are positioned within the predicted primary footprint to capture potential accumulation areas, and along the Zone 2 boundary (northwest–southeast) to detect the outer extent of farm effects.
- **Beyond Zone 2 stations** (teal) are positioned at the OLE boundary and the outer edge of Beyond Zone 2 to assess the extent of farm-related enrichment effects. Ecological effects are generally not expected.
- **Reference stations** (note that only one indicative location is shown Figure 4, indicated in dark blue) are used to compare farm effects to background levels outside of the farm influence.

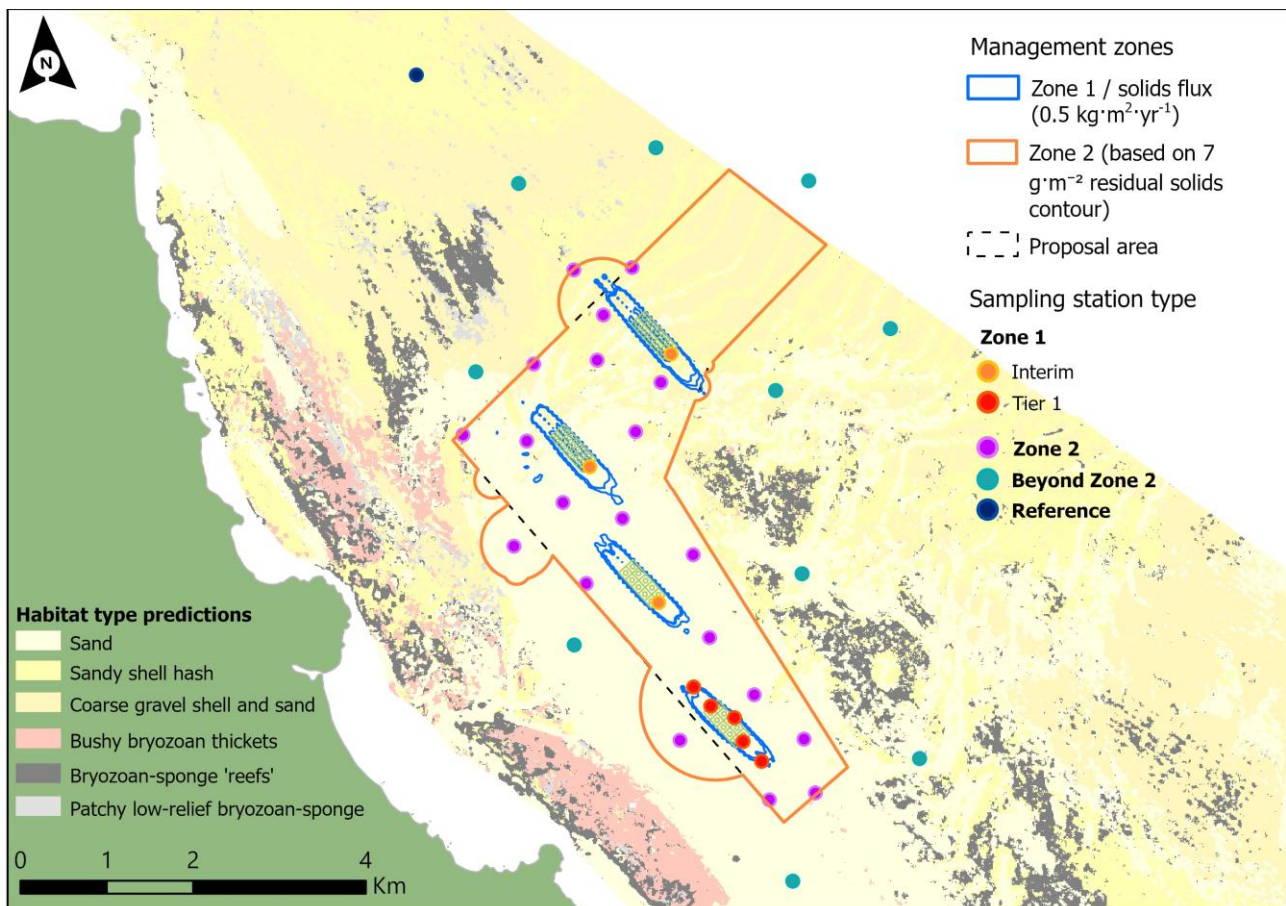


Figure 4. Indicative monitoring stations and sampling type required for Zone 1, Zone 2 and Beyond Zone 2 at peak feed input for Farm 1A. Indicative reference station location shown for illustrative purposes; the number and final placement of reference stations will be defined through baseline monitoring. Zone boundaries have been defined based on Stage 1 depositional modelling (see Figure 2).

4.3 Replication

Triplicate¹² sediment samples should be collected from each sampling station and processed throughout the baseline survey period and until each farm block has completed a full production cycle. These samples should capture small-scale variability under all potential enrichment scenarios. Once sufficient data have been processed to assess variability and / or demonstrate that observed farm effects remain within predicted ranges, the need to continue processing all replicates should be reviewed in consultation with experts, and sampling effort may be reduced accordingly for the remainder of Stage 1 monitoring. The operator may also elect to sample more intensively than the design outlined here if finer-resolution information on enrichment is required.

¹² Three replicates are proposed initially; however, additional replication may be required if higher variability is detected during baseline surveys.

The final survey design and replication strategy should be refined following assessment of baseline data to ensure that key sources of variability are adequately captured. Where baseline data indicate higher-than-expected variability in key indicators, additional sampling may be warranted to reduce the likelihood of natural variation being misinterpreted as a farm-related effect. Similarly, where pronounced stratification within Zone 2 is identified, additional reference stations may be established to provide appropriate context and ensure adequate representation within each stratum. The monitoring framework would then test for effects within each stratum, using reference conditions as the basis for compliance assessment.

Where statistical testing is applied within the initial environmental quality standards (iEQS) framework, power analysis using baseline data should be undertaken to confirm that replication and station numbers are sufficient to robustly assess farm-related effects with EQS. A follow-up power analysis once the farm is operational may also be appropriate once variability at impacted sampling stations has been established. The selected analytical approach (e.g. hypothesis testing or alternative framework), along with the process and justification for determining replication, should be documented in the EMOP (Giles et al. 2021).

At least five seabed images should be captured from video / still images at each station for assessment of epifaunal indicators, bacterial cover and any other relevant observations.

To give an overview of sampling effort required across all survey types, the station and replicate numbers are given in Table 2.

Table 2. The number of sampling stations (Stations) and replicates (Reps) for each station and survey type during Stage 1 monitoring. The number of samples processed (Sample no.) required for each survey is also given. Note that fewer samples will be collected in some cases.

Zone and station type	Baseline (pre-farming)			Stage 1		
	Stations	Reps	Sample no.	Stations	Reps	Sample no.
Zone 1						
Before stocking	1 / farm*	3	12	–	–	–
Farm at peak production	–	–	–	5**	3	15
Interim farms (3)	–	–	–	1 / farm	3	9
Zone 2						
	20	3	60	20	3	60
Beyond Zone 2						
	10	3	30	10	3	30
Reference						
	10	3	30	10	3	30
<i>Total samples (minimum)</i>			132			144

*Assumes only one anticipated pen edge station is sampled in triplicate during baseline monitoring.

**Assumes five pen stations (three at the pen edge and two at the Zone 1 boundary) are sampled in triplicate during Stage 1 monitoring.

4.4 Environmental quality standards for sandy seabeds

Extending existing indicators to novel habitat types presents challenges, as environmental variables often exhibit site-specific responses, and natural baseline values can vary substantially among locations as a function of local hydrodynamics and other physical or geographical factors (Keeley et al. 2012, 2013). A series of parameters are proposed as *i*EQS, adopting soft sediment standards from other areas in the absence of historical farming data for sandy sediments. It is anticipated that EQS for open ocean aquaculture sites will need to be developed on a site-specific basis, particularly during the early stages of industry development in Aotearoa New Zealand (Giles et al. 2021). These *i*EQS therefore represent a starting point within an adaptive framework, recognising that the most appropriate and practicable indicators will evolve as empirical evidence accumulates and new monitoring technologies emerge.

The goal of the EQS development process is to establish robust and reliable quantitative standards. However, the inherent uncertainty in how farming effects will manifest in new environments precludes the ability to define such thresholds confidently at the outset. Accordingly, a precautionary approach is warranted, where conservative thresholds for existing indicators used elsewhere will be applied initially and refined progressively as empirical data become available and site-specific responses are better understood. Some parameters commonly used in the Marlborough Sounds (e.g. b-MBI and AMBI) will require validation to the ecological conditions at the Hananui proposal area to confirm their relevance and sensitivity.

The *i*EQS are set according to descriptions of progressive stages of enrichment defined in the ES framework used in the Marlborough Sounds (Table 3). The proposed sampling plan aims to establish indicator responses across a range of conditions (e.g. from natural to most impacted¹³), providing a dataset from which indicator importance can be weighted, and thresholds can be checked and recalibrated if necessary to facilitate the transition from *i*EQS to EQS.¹⁴

The survey design proposed in this report will support this process by establishing indicator responses across a range of conditions (from natural to most impacted), thereby providing a dataset from which thresholds can be reviewed and recalibrated as needed to facilitate the transition from *i*EQS to EQS.

¹³ Note that additional validation will likely be appropriate at later stages as predicted enrichment levels increase.

¹⁴ This stepwise approach is consistent with best practice for novel habitat types and was applied in the Blue Endeavour application by The New Zealand King Salmon Company Ltd.

Table 3. General description and main environmental characteristics of Enrichment Stages (ES) 1–7, adapted from Fletcher et al. (2022).

ES	General description
1	Very low productivity – Environmental variables comparable to an unpolluted / unenriched pristine reference site.
2	Minor enrichment / enhanced zone – This can also occur naturally or from other diffuse anthropogenic sources. Taxa richness usually greater than for the reference site. Minor increases in animal abundance possible.
3	Moderate enrichment – Coupled with a significant change in community composition. Notable abundance increase, richness and diversity usually lower than for the reference site. Opportunistic and tolerant species (e.g. capitellids, dorvilleids) begin to dominate. Sediment chemistry may show slight deteriorations, and <i>Beggiatoa</i> -like bacteria may be visible in patches.
4	High enrichment – A transitional stage between moderate effects and peak macrofaunal abundance. A major change in community composition is evident. Opportunistic species dominate, but other taxa may persist. Major sediment chemistry changes (approaching hypoxia), and patches of <i>Beggiatoa</i> -like bacteria likely to be visible.
5	Very high enrichment – Sediments are highly enriched and macrofauna are at peak abundance. Total abundances can be extreme. Diversity usually significantly reduced, but moderate richness can be maintained. Sediment organic content usually slightly elevated. <i>Beggiatoa</i> -like bacteria may form visible 'mats', and sediment outgassing is possible.
6	Excessive enrichment – Transitional stage between peak abundance and azoic conditions (no infauna present). This has not previously been observed at high-flow salmon sites in the Marlborough Sounds.
7	Severe enrichment – Anoxic and azoic; sediments no longer capable of supporting macrofauna. Organic material accumulating in the sediments. This has not previously been observed at high-flow salmon sites in the Marlborough Sounds.

Suggested parameters for use in the *i*EQS are those that indicate where a soft sediment community sits along the gradient of organic enrichment:

- **Sediment sulphide concentrations (total free sulphides, S^{2-}):** Sulphides can build up in sediments where excess organic matter is broken down by microbes under conditions of insufficient oxygen. They can be toxic to sediment-dwelling animals, particularly in association with anoxic conditions.
- **Redox / pH index:** Redox (reduction–oxidation) potential (Eh) indicates the availability of electron acceptors in the environment to allow for microbial breakdown of organic matter. Values in the marine environment typically range between +400 mV in surface waters and -200 mV for anoxic sediments. pH normally varies between 8.0 and 8.1 in surface water, down to 7.0 in anoxic water and sediments, and can fall below 6.5 in strongly anoxic sediments. An index derived from redox and pH has been developed for management of salmon farming in Aotearoa New Zealand and Norway: higher acidity (lower pH) and lower redox potential yield higher index values (Figure 5).
- **Bacterial Metabarcoding Biotic Index (b-MBI):** An index that uses bacterial environmental DNA (eDNA) for quantifying benthic organic enrichment (Fletcher et al. 2022). The b-MBI is calculated based on the presence of bacterial indicators, which have been assigned to an Eco-Group (EG) with an associated weighting. Results range from 1.69 (pristine) to 8.5 (capped at 7, severe

enrichment). A baseline eDNA survey of the Hananui aquaculture site was undertaken in October 2021 and suggests that the b-MBI approach is a promising tool for monitoring of the site. The results of this survey are presented in Cawthron Advice Letter 2237 (Appendix 1). Incorporation of this index at the Hananui proposal area would require validation against infaunal data over at least 2 years¹⁵ and therefore would not be immediately available for use as an *i*EQS.

- **Near-seabed water column dissolved oxygen (DO):** Decreases in DO can be caused by increases in biological activity, including bacterial breakdown of excessive organic matter. Near-seabed reductions in DO can therefore indicate high biological activity on the seabed.
- **AZTI's Marine Biotic Index (AMBI):**¹⁶ An index calculated from seabed communities. Animal species or taxa are categorised into ecological groups, according to their sensitivity (or tolerance) to environmental stress, such as enrichment. The distribution of ecological groups in a community is then used to calculate the index (AMBI) of 'Ecological Quality' (Borja et al. 2000). Index values are between 0 (normal) and 6 (extremely disturbed).
- **Benthic Quality Index (BQI):**¹⁷ A marine BQI used to classify sediments based on established species tolerance values (Rosenberg et al. 2004) has been used as a reliable indicator of organic enrichment in the Marlborough Sounds for several years and will require development and validation to the ecological conditions specific to the Hananui proposal area. BQI ranges from 0 (highly impacted) to 20 (reference conditions).
- **Other biotic indices, such as total abundance (N), taxa richness (S), Shannon–Wiener diversity (H'), Margalef's d and Pielou's J',** will be used collectively with expert judgement with reference to established responses to the enrichment gradient from other regions, as per Keeley et al. (2012). We note that infaunal samples at some stations may only yield abundance and taxonomic diversity as infaunal communities are naturally too depauperate to reliably use more complex calculated community metrics in some areas.
- **White, anaerobic bacterial coverage:** Extensive coverage of sulphide oxidising bacteria can be an indicator of excessively enriched, anaerobic sediments (Fletcher et al. 2022). However, it has also been observed that seabed communities under bacterial mats are capable of assimilating organic wastes (e.g. McGrath et al. 2020). This is therefore an appropriate component of monitoring but should not be used as a standalone indicator (see also, Hamoutene 2014).
- **Outgassing from sediments:** This can occur when hydrogen sulphide or methane gas is produced through the breakdown of organic matter under anaerobic conditions; the permitted level of impact is set below the threshold at which such effects would occur.

¹⁵ More time may be necessary to capture interannual variation.

¹⁶ May not be possible to calculate at some sites due to naturally depauperate infauna.

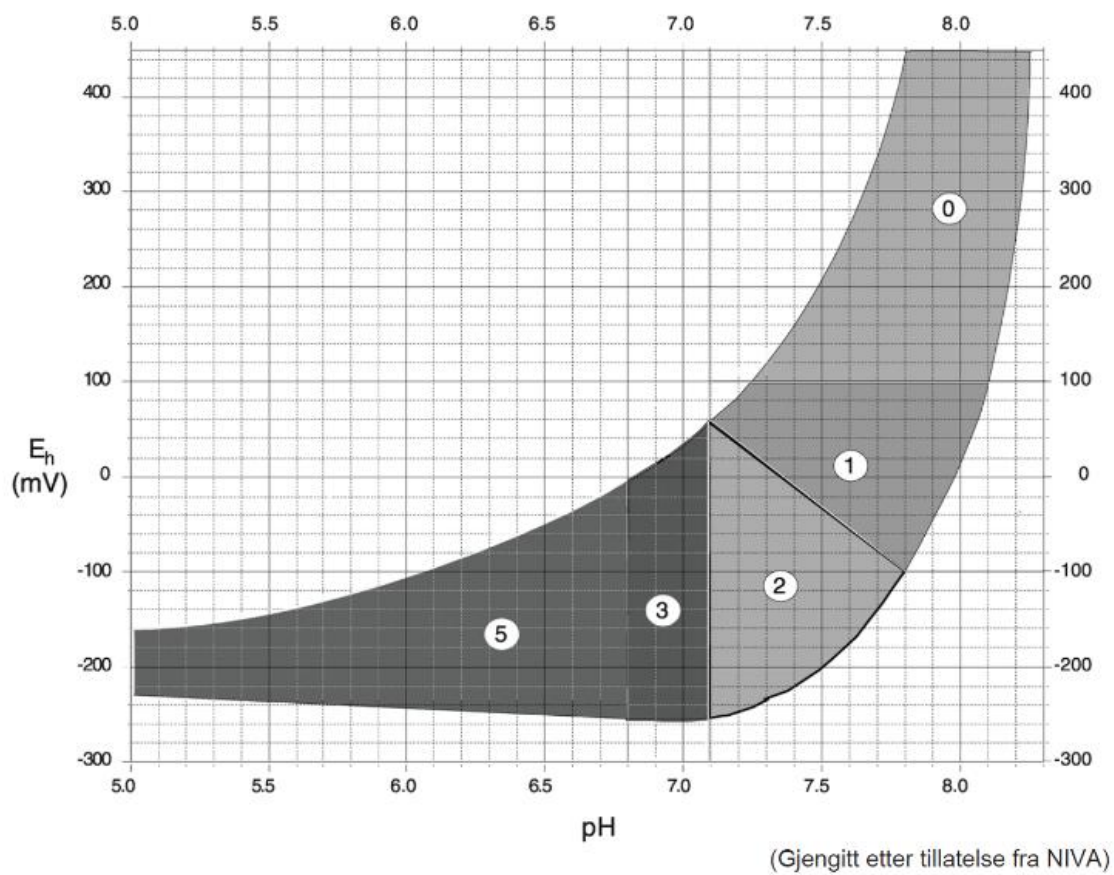


Figure 5. Redox – pH classification plot. Source: Appendix D, Norwegian Standard NS 9410:2016 (Standards Norway 2016).

Table 4. Guiding environmental principles, initial-EQS and response triggers for sandy habitats. Tier 1 indicators are to be used routinely to assess environmental impact and, when breached, should trigger Tier 2 surveys. S^{2-}_{UV} = total free sulphides, UV = ultraviolet (spectrophotometry) method, b-MBI = bacterial Metabarcoding Biotic Index, DO = dissolved oxygen, AMBI = AZTI's Marine Biotic Index, BQI = Benthic Quality Index, N = total abundance, S = taxa richness, H' = Shannon–Wiener diversity index, d = Margalef's diversity index, J' = Pielou's evenness.

Guiding principles – sandy habitats	Initial-EQS (iEQS) indicators and associated thresholds	Response trigger
Zone 1		
Peak production: full-suite monitoring		
Farm shall not become very highly enriched (all areas < cf. ES 5)	<p>iEQS for identifying very high enrichment (ES 5):</p> <ul style="list-style-type: none"> Quantitative indicators (station average): <ul style="list-style-type: none"> $S^{2-}_{UV} > 800 \mu\text{M}$ or standard method Redox / pH index > 3.0 b-MBI > 4.5 Macrofaunal indicators (AMBI > 5.1, BQI < 2.6, expert judgement that other available indicators [e.g., N, S, H', J' and d] indicate very high enrichment) Station average near-bottom water column DO $< 90\%$ reference White, anaerobic bacterial coverage $> 50\%$. Visible free outgassing from sediments. 	If two or more iEQS indicators are triggered, farm management response actions, as outlined in the adaptive management plan, should be initiated.
Interim stations: tiered monitoring		
Farm shall not become very highly enriched (all areas < cf. ES 5)	<p>Tier 1 iEQS for identifying very high enrichment (ES 5):</p> <ul style="list-style-type: none"> Quantitative indicators (station average): <ul style="list-style-type: none"> $S^{2-}_{UV} > 800 \mu\text{M}$ or standard method / in tandem for a while Redox / pH index > 3.0 Station average near-bottom water column DO $< 90\%$ reference White, anaerobic bacterial coverage $> 50\%$ Visible free outgassing from sediments. <p>Tier 2 iEQS for identifying very high enrichment (ES 5, archived):</p> <ul style="list-style-type: none"> Quantitative indicators (station average): <ul style="list-style-type: none"> b-MBI $> 4.5^{17}$ 	Tier 2 is triggered when any two or more Tier 1 indicators are triggered at any station or outgassing is observed; archived Tier 2 samples should be processed. If Tier 2 indicators are exceeded, farm management response actions, as outlined in the adaptive management plan, should be initiated.

¹⁷ Incorporation of this index at the Hananui proposal area would require validation against infaunal data over at least 2 years before applicability as a Tier 1 iEQS indicator.

Guiding principles – sandy habitats	Initial-EQS (iEQS) indicators and associated thresholds	Response trigger
	<ul style="list-style-type: none"> Macrofaunal indicators (AMBI > 5.1, BQI < 2.6, expert judgement that other available indicators [e.g., N, S, H', J' and d] indicate very high enrichment). 	
Zone 2		
Zone 2 shall not become highly enriched (all areas < cf. ES 4)	Tier 1 iEQS for identifying high enrichment (ES 4): <ul style="list-style-type: none"> Station average: <ul style="list-style-type: none"> $S^{2-UV} > 500 \mu M^{18}$ b-MBI > 4.¹⁹ 	Tier 2 should be triggered when either or both Tier 1 indicators are exceeded at any Zone 2 station.
	Tier 2 iEQS for identifying high enrichment (ES 4): Quantitative macrofaunal indicators (station average): <ul style="list-style-type: none"> AMBI > 4.4* BQI < 4.0* Expert judgement that other available indicators (e.g., N, S, H', J' and d) indicate high enrichment. 	Farm management response should be initiated when Tier 2 indicators are triggered (AMBI, BQI, expert judgement) at any Zone 2 station. <i>If only expert judgement available, indications of high enrichment = farm response action required. If AMBI and BQI can be calculated, then 2/3 indicators triggered = farm management response triggered.</i>
Beyond Zone 2		
Beyond Zone 2 shall not become moderately enriched (all areas < cf. ES 3) Minor enrichment permitted but not sufficient to cause ecological degradation or loss of biodiversity	Tier 1 iEQS for identifying moderate enrichment (ES 3): <ul style="list-style-type: none"> Station average: <ul style="list-style-type: none"> $S^{2-UV} > 250 \mu M$ or, > upper 95% CI value for relevant reference stations b-MBI > upper 95% CI value for relevant reference stations.¹⁹ 	Tier 2 should be triggered when either or both Tier 1 indicators are exceeded at the Beyond Zone 2 stations.
	Tier 2 iEQS for identifying moderate enrichment (ES 3): Quantitative macrofaunal indicators (station average): <ul style="list-style-type: none"> AMBI > upper 95% CI for relevant reference stations BQI < lower 95% CI for relevant reference stations Expert judgement that other available indicators (e.g., N, S, H', J' and d) indicate moderate enrichment. 	Farm response actions should be initiated when Tier 2 indicators are triggered (AMBI, BQI, expert judgement) at any of the Beyond Zone 2 stations. <i>If only expert judgement available, indications of moderate enrichment = farm response action required. If AMBI and BQI can be calculated then 2/3 indicators triggered = farm management response triggered.</i>

¹⁸ Point equivalent to approximately the transition between 'Moderate' and 'Poor' status based on international data published by Fisheries and Oceans, Canada, and Cranford et al. (2020), and as recommended in the Aquaculture Stewardship Council benthic monitoring whitepaper (ASC 2022). Assumes use of the UV spectrophotometry of sediment pore water method.

5. Biogenic habitats

The farm layout has been designed to minimise deposition of farm-derived material on biogenic habitats. While low-level inputs may occur through resuspension, far-field deposition and accumulation are expected to be minimal and may not be readily detectable (Bennett et al. 2025). Biogenic habitat monitoring is a precautionary measure, designed to provide early detection of an effect if it arises, through an approach that employs a broad range of monitoring tools. Monitoring design will be refined by recommendations made in the baseline EMOP.

5.1 Assessment framework

Given the limited precedent for organic enrichment effects on key taxa and the time required for such effects to become detectable, establishing EQS for biogenic habitats at this stage would be premature. Instead, potential adverse impacts to ecosystem function should be assessed through multiple lines of evidence relative to background conditions and over time, allowing farm-related effects to be distinguished from natural variability and changes in individual indicators to be interpreted in the context of broader ecosystem change. Any significant changes potentially attributable to the farm¹⁹ would trigger full quantitative analysis to determine causality, with a management response initiated where warranted. Key taxa identified in the context of the biogenic habitat around the Hananui site are sponges and reef-building bryozoans (Bennett et al. 2025). These species have high ecological importance, and reductions in these taxa may cause cascading ecological effects. Additional key taxa for monitoring will be identified through expert judgement following baseline surveys.

Tiered monitoring frameworks are most effective once effects are well characterised (Giles et al. 2021). For the Hananui proposal area, intensive baseline monitoring in the early stages will provide data to target the monitoring indicators, refine the methodological approach and address uncertainties surrounding impacts of organic enrichment. The baseline survey is a particularly important stage in monitoring programme development for these relatively poorly understood biogenic habitats. The monitoring and management framework must remain adaptable over time to facilitate the inclusion of new information and technology as they become available. This may include both emerging research / technology and validation of methodologies used in other regions of Aotearoa New Zealand.

5.2 Sample approaches

Habitat mapping

Habitat mapping will track large-scale changes in biogenic habitat structure²⁰ over time, providing a system-level view of potential impacts. Methods may include multibeam echosounder or side-scan

¹⁹ E.g. those observed at impact sites but not at reference sites.

²⁰ Repeating surveys must be consistent in scale and resolution to ensure data is comparability over time.

sonar surveys, with multibeam echosounder recommended where sufficient resolution is required to quantify differences between surveys. The survey extent required will be determined during the baseline surveys.

Video surveys

Biogenic habitat monitoring locations can be informed using pre-existing data, including seabed imagery from habitat surveys, maps from predictive habitat modelling and results from Stage 1 depositional modelling (Bennett et al. 2025). Indicative survey locations are illustrated in Figure 6. These will be refined during baseline data collection.

Spatial variability in biogenic habitats is generally high, and to reliably capture change over time, survey design often relies on the ability to repeatedly sample the same transects and the communities within them with high spatial accuracy. Where this cannot be achieved, sampling intensity will be increased to account for the higher variability, ensuring that potential adverse effects on biogenic habitats can still be detected early.

Replicate and station number requirements for video surveys will be confirmed through a power analysis using baseline survey data to ensure replication is appropriate to effect-size detection. A minimum of five transects targeting biogenic habitats should be surveyed per biogenic sampling site (indicated by circles in Figure 6), each at least 100 m in length. Reference stations must host communities that are ecologically similar to those being monitored within the Beyond Zone 2 area, providing a valid basis for detecting potential farm-related effects. Station numbers and transect placement may be refined adaptively as baseline data are analysed to build a statistically robust and sensitive monitoring design.

Soft sediment sentinel monitoring stations should be placed at the boundaries of biogenic habitats²¹ to provide early warning if organic enrichment approaches habitat edges. This will also help determine whether observed changes in biogenic habitats are caused by farm activities.²²

²¹ Where soft sediment sentinel stations overlap with Beyond Zone 2 sandy sediment monitoring stations, the same station may be used for both purposes, based on expert judgement.

²² For example, using farm-waste tracers such as terrestrial fatty acids to verify the presence of salmon waste.

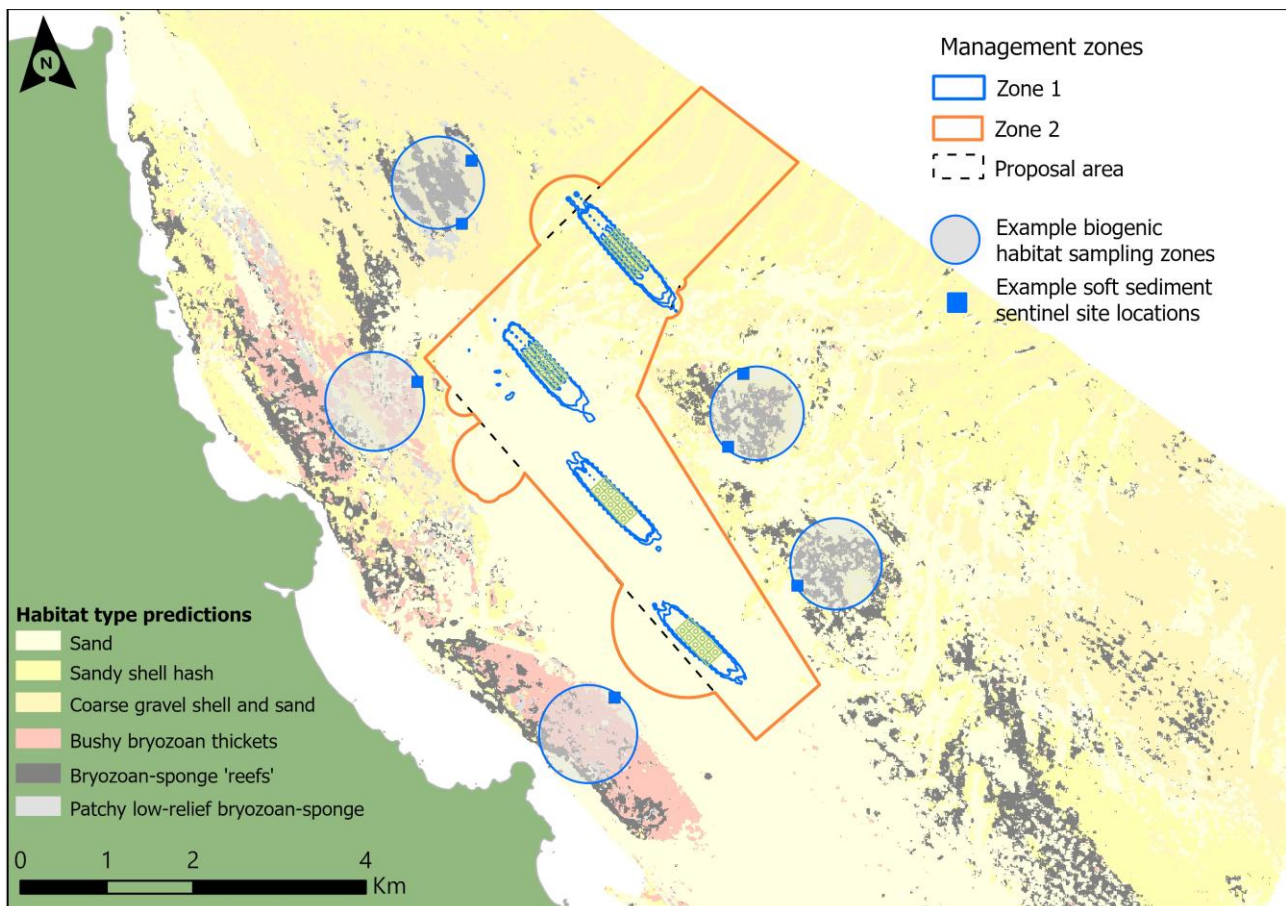


Figure 6. Indicative example of potential biogenic habitat sampling locations, where repeat transects may be established, with associated soft sediment sentinel stations in the Beyond Zone 2 area.

5.3 Survey timing

Biogenic habitat monitoring will be conducted in tandem with sandy seabed sampling. During Stage 1, surveys will occur approximately every 6 months for the first 2–3 years while the site is developed, depositional effects footprints are validated and system responses are clarified. Once sufficient data indicate that farm-related effects to biogenic habitats are minimal, the monitoring frequency may be reduced²³.

5.4 Indicators for identifying adverse effects to ecosystem function

Data collected from habitat mapping, such as changes in habitat extent (m²), structural complexity (rugosity) and seafloor composition (the proportion of different substrate types), all provide indications

²³ This process will be outlined in the EMAP and EMOP and will likely require agreement with expert opinion and Environment Southland.

of possible deviation from background conditions (Table 5). While these data can reveal signs of ecological deterioration from background conditions, they may also reflect non-adverse shifts in ecosystem structure and function as a result of farm activities. These surveys should be complemented by finer-scale assessments of key taxa and community composition, allowing both broad ecosystem trends and localised organism-level responses to be monitored relative to baseline and reference conditions.

Video imagery surveys are a non-destructive method. The primary indicators should be percentage cover of key taxa and changes in community structure, both assessed using a beyond before–after, control–impact (BACI)-type approach. This approach will quantify changes both over time (e.g. to baseline data) and relative to appropriate reference stations. Assessing percentage cover of key taxa alongside community structure is a multi-evidence approach that evaluates different aspects of ecosystem function. Expert judgement should be used to determine whether conditions have deteriorated from background, ensuring that potential adverse effects are identified early. If deteriorations are evident, a second tier of investigation can be supported by additional sampling through various lines of evidence (e.g. farm-waste tracers) to investigate the likely cause (e.g. rule out or attribute to farm activities).

In addition, taxa-specific observations may provide useful context for assessing communities, such as recruitment of new colonies, overall condition (presence and extent of living tissue) and presence of disease or notable areas of growth.²⁴ It may also be useful to collect physical samples (if this can be done without causing unacceptable disturbance). Physical samples would allow analysis of additional parameters to relate organismal and community responses to farm activities. These samples would also provide baseline data for developing new EQS for biogenic habitats and ensure accurate species identification for subsequent image-based analyses and sensitivity assessments.

The types of physical samples that may be collected include:

- flocculent material overlying biogenic habitats (e.g. using a specialised sampling device such as a Substrate-Independent Benthic Sampler²⁵ [SIBS]; Keeley et al. 2025), to determine composition of bacterial communities (using 16S eDNA) or look for evidence of farm waste (e.g. using fish-waste markers) in overlying sediments.
- soft tissues of fauna to:
 - determine presence of farm-waste signatures (e.g. terrestrial fatty acid markers)
 - provide early warning signs of stress in key taxa.²⁶

²⁴ Abigail Smith, as per expert evidence: <https://www.epa.govt.nz/assets/Uploads/Documents/Fast-track-consenting/Hananui/Amended-Hananui-decision-22-August-2023.pdf>

²⁵ While the methodology detailed in Keeley et al. (2025) demonstrated success in sampling hard-bottom habitats, it may require modification to account for local conditions. While such capability is not currently available in Aotearoa New Zealand, its future adaptation represents a valuable direction for method development.

²⁶ Further research is required to elucidate the response of key taxa to farm-derived enrichment and to ensure monitoring approaches remain aligned with evolving scientific understanding and best practice. The EMOP should highlight this and any other supplementary investigations that address key uncertainties and inform the development of novel EQS for biogenic habitats.

Samples from soft sediment sentinel monitoring stations (located in sandy seabed environments on the boundaries of the biogenic habitat) should be routinely analysed for Tier 1 *i*EQS for identifying moderate enrichment (as in Table 4). Data from these stations can (a) provide early warning if organic enrichment is measured close to the biogenic habitat boundary and (b) help determine whether any observed changes within the biogenic habitat may be linked to farming activities. Advances in surface sampling technologies (e.g. SIBS-type devices) may eventually negate the need for separate sentinel stations by enabling direct, non-destructive sampling within biogenic habitats. Continued method development in this area is encouraged to strengthen monitoring capability across habitat types.

Table 5. Guiding environmental principles, indicators for identifying adverse effects to ecosystem function and response triggers for biogenic habitats.

Guiding principles – biogenic habitats	Indicators for identifying adverse effects to ecosystem function	Response trigger
No adverse effect sufficient to disrupt ecosystem function	<p>Video footage (video and still imagery) analyses</p> <ul style="list-style-type: none"> • Quantitative: <ul style="list-style-type: none"> ○ Statistically significant change in the percent cover of key taxa relative to appropriate reference station(s)²⁷ ○ Statistically significant change in community structure (positive or negative) relative to appropriate reference station(s).¹⁷ • Qualitative <ul style="list-style-type: none"> ○ Presence of any fish-farm derived material ○ Evidence of physical disturbance. <p>Habitat mapping</p> <ul style="list-style-type: none"> • Statistically significant changes in the total area of habitat extent (m²), structural complexity (rugosity), and / or seafloor composition (proportion of different substrate types) over time.¹⁷ <p>Soft sediment sampling (sentinel stations)</p> <p>Tier 1 iEQS for identifying moderate enrichment (ES 3):</p> <ul style="list-style-type: none"> ○ $S^2_{UV} > 250 \mu M$ or, > upper 95% CI value for relevant reference stations ○ b-MBI > upper 95% CI value for relevant reference stations. <p>Tier 2 iEQS for identifying moderate enrichment (ES 3):</p> <p>Quantitative macrofaunal indicators</p> <ul style="list-style-type: none"> ○ AMBI > upper 95% CI for relevant reference stations ○ BQI < lower 95% CI for relevant reference stations ○ Expert judgement that other available indicators (e.g., N, S, H', J' and d) indicate moderate enrichment. 	<p>If a statistically significant change in cover for key taxa or community structure is detected based on the BACI design and in conjunction with farm activities, farm management actions should be initiated.</p> <p>Evidence of physical disturbance should be evaluated using expert judgement alongside other indicators, such as farm-waste tracers, to determine whether the farm or external forces (e.g. ship anchoring, oyster dredging) are the likely source.</p> <p>If a statistically significant change in these parameters is detected based on the BACI design, farm management actions should be initiated.</p> <p>If either or both Tier 1 indicators are triggered, expert judgement should be required to assess whether there is evidence of biogenic habitat impact before proceeding to Tier 2.</p> <p>If Tier 2 indicators are triggered (AMBI, BQI, expert judgement) at any of the Beyond Zone 2 stations, farm management response should be initiated.</p> <p><i>If only expert judgement available, indications of moderate enrichment = farm management response triggered. If AMBI and BQI can be calculated, then 2/3 indicators triggered = farm management response.</i></p>

²⁷ Implies the use of a beyond BACI approach to test for a significant control vs impact interaction term. The same statistical design will be applied for univariate (change in the percentage cover of key taxa, total area of extent, habitat extent, rugosity) and multivariate analyses (change in community structure, seafloor composition).

6. Requirements for environmental monitoring and management plans

This report provides recommendations for the environmental monitoring and management approach for seabed effects at the Hananui proposal area. As outlined in Section 3, prior to any baseline sampling, a baseline EMOP will need to be developed and certified to establish pre-operational conditions and inform subsequent monitoring phases. It is also expected that the recommendations provided in our report will help guide the development of the EMOP document. The EMAP and EMOP will then be developed at a later stage by suitably qualified and experienced providers and will incorporate stakeholder feedback, address any refinements to the farming proposal, and provide the detailed framework for seabed monitoring and adaptive management. It is noted that the sub-sections assume two documents; however, a single document covering both the EMAP and EMOP requirements would be acceptable. The following sub-sections detail the requirements for each of these plans.

6.1 Environmental management plan

The EMAP will define the framework for monitoring and adaptive management of marine farming activities at the Hananui aquaculture site.

The EMAP must, at a minimum, include the following:

- **Farm layout and operations:** Plans showing the location of each marine farm, with details of stocking densities, progressive feed discharge rates and the fallowing sequence for each farm.
- **Monitoring design and implementation:** A clear process for selecting appropriate indicators, sampling locations and analytical methods, and for determining the appropriate sampling intensity required to detect and assess effects of farm activities on both sandy and biogenic habitats.
- **Assessment and evaluation:** Procedures for assessing monitoring results against predicted environmental effects and the suggested iEQS for sandy seabeds to evaluate whether environmental outcomes remain within acceptable limits.
- **Model validation and review:** A framework for reviewing and validating depositional modelling and specifying re-modelling requirements to inform progression between development stages.
- **Response to EQS threshold exceedance or adverse effects:** Farm management procedures for responding to monitoring results that indicate EQS exceedance or the occurrence of significant adverse environmental effects.
- **Farm relocation or adjustment:** A process for evaluating and implementing changes in farm positioning within the consented area where required to avoid or mitigate adverse effects.
- **Stage progression and certification:** A process for proposing progression between development stages, including independent review by suitably qualified and experienced providers and certification by Environment Southland.

- **EQS refinement:** An iterative process for refining EQS for sandy and biogenic habitats, informed by monitoring data and emerging empirical evidence to improve alignment with observed environmental responses.
- **Review and continuous improvement:** A process for the periodic review of the EMAP to ensure alignment with evolving best practice in finfish aquaculture, and advances in monitoring, management and environmental impact assessment methodologies.

6.2 Environmental monitoring plan

The objective of the EMOP is to define the methodology and processes necessary to meet the monitoring requirements of the consent and ensure that monitoring allows robust assessment of environmental response according to consent conditions. The EMOP should also maintain alignment with evolving best practice monitoring methods and parameters for the site.

The EMOP must contain the following:

- **Monitored effects:** Identification of the environmental effects and response variables to be monitored.
- **Monitoring locations:** Specification of impact and reference site locations, consistent with the monitoring design outlined in the baseline EMOP.
- **Indicators and parameters:** Identification of the biological, chemical and physical indicators to be measured to assess seabed condition and enrichment.
- **Sampling design:** Description of the timing, frequency, methods and level of replication for all sampling activities, ensuring adequate spatial and temporal resolution.
- **Analytical methods:** Specification of analytical techniques to be applied for data processing and interpretation.
- **Reporting:** Requirements for reporting formats, timing and content to ensure transparency, traceability and consistency of monitoring outcomes.
- **Plan review and adaptation:** A process for periodic review of the EMOP, including procedures for updating methodologies, adjusting sampling intensity or expanding monitoring scope where results indicate this is necessary.

7. Progression between stages

Progression to Stage 2 farming would entail the addition of a second block of 10 pens per farm site (B blocks), which would result in an increased feed discharge to 25,000 tonnes per year. Movement between stages can be justified on an environmental basis when it can be shown that farm activities are not likely to result in unacceptable environmental effects. Progression between stages must therefore meet the following requirements:

- At least two full production cycles have been completed at Stage 1 feed levels for each farm or block.
- Monitoring results confirm that environmental effects are consistent with, or less than, those predicted for Stage 1 and are not likely to result in unacceptable adverse effects.
- The environmental effects of farm operations across the site and over time have been evaluated by suitably qualified and experienced providers and determined to remain within acceptable limits.

A depositional model validation exercise may also be undertaken prior to progressing to Stage 2, particularly where updated information would help confirm the predicted extent and magnitude of effects. At a minimum, this would require re-running the depositional modelling using actual feed inputs to confirm that observed environmental effects at Stage 1 are less than, or equal to, those predicted by the original modelling. If this exercise was to demonstrate that farm-derived environmental effects were worse than predicted, investigation into why the model results do not align should be undertaken. The model can then be updated so that it aligns with the observed effects. Finally, Stage 2 would be re-modelled using a validated version of the model and reassessed.

Appendix 1. Cawthron Advice Letter 2237



20 May 2022

Thomas Hildebrand
Ngāi Tahu Seafood Limited
6 Bolt Place / PO Box 3787
Christchurch, New Zealand

ID: 2237

Kia ora Thomas

Re: First baseline environmental DNA survey of the Hananui aquaculture site

From research conducted over the last ten years, the Cawthron Institute (Cawthron) has demonstrated the feasibility of using bacterial environmental DNA (eDNA) for quantifying benthic organic enrichment under salmon farms^{1,2,3,4}. We have demonstrated that the eDNA-derived variable 'bacterial Metabarcoding Biotic index' (b-MBI), could be used in place of the macrofauna component to calculate Enrichment Stage (ES). ES is the metric currently used in assessing compliance with benthic standards for several salmon farms in the Marlborough region. Our research has produced a refined, robust, and defensible 'Overall molecular ES' index that operates on the same compliance threshold scale as the 'Overall traditional ES'. As a result, agreement has been reached to incorporate the eDNA tool into the benthic Best Management Practice (BMP) guidelines for Marlborough. This required the development of a Standard Operating Protocol (SOP) for the collection, isolation, and processing of samples (completed in June 2021), and sequencing of additional sediment samples to further validate the tool (completed in November 2021; see results detailed in Pochon et al. 2021b⁵).

We understand Ngāi Tahu Seafood Resources Ltd (Ngāi Tahu Seafood) are interested in using the eDNA tool in environmental monitoring of the proposed Hananui open ocean aquaculture site offshore of northern Stewart Island / Rakiura. Cawthron were contracted by Ngāi Tahu Seafood to conduct a baseline eDNA survey to assess the potential for

¹ Keeley N, Wood SA, Pochon X 2018. Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. *Ecological Indicators*, 85: 1044-1057.

² Pochon X, Wood S, Atalah J, Laroche O, Zaiko A, Keeley N 2020a. A validated protocol for benthic monitoring of New Zealand's salmon farms using environmental DNA. Prepared for Seafood Innovation Ltd, New Zealand King Salmon Company Ltd, Ministry for Primary Industries and Marlborough District Council. Cawthron Report No. 3400. 51 p. plus appendices.

³ Pochon X, Atalah J, Fletcher L 2020b. Validation of a bacterial molecular biotic index (b-MBI) from 2019-2020 fish farms data. Cawthron Letter 2051.

⁴ Pochon X, Atalah J, Fletcher L, Laroche O, Elvines D, Zaiko A, Wood SA, Keeley N 2021a. A molecular index refined for benthic monitoring of salmon farms in the Marlborough Sounds. Prepared for Benthic Standards Working Group (Marlborough Sounds). Cawthron Report No. 3602. 25 p. plus appendices.

⁵ Pochon X, Atalah J, Fletcher L 2021b. Final validation of 'Overall molecular ES' from 2020-2021 farm data. Cawthron Letter 2188.

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application of the b-MBI index for future monitoring of benthic impacts at the site. In this first environmental survey, we (1) explored the diversity of bacterial assemblages naturally occurring across the Hananui site, (2) compared the bacterial community composition recorded at the Hananui site with a previously published bacterial dataset¹ obtained from salmon farm activities in nearby Big Glory Bay (BGB), and (3) calculated b-MBI values for seven sampling stations across the Hananui site using the existing GeneCodeID database.

The Ministry for Primary Industries (MPI) have given permission for the release of two Cawthron letters^{3,5} describing the validation of the eDNA technique/tool to Ngāi Tahu, as this new dataset aligns with previous data reviewed by Benthic Standards Working Group (BSWG) members and international experts in January 2018 and February 2020. We note that the final recommendations presented in Pochon et al. (2021b⁵) have yet to be reviewed by the BSWG.

Methods

Sediment samples were collected from 'sandy seabed' environments across the Hananui site on 7-8 October 2021. Triplicate sediment samples were collected from seven sampling stations (C1, G1, G25, G29, G30, G31 and G32; 21 samples in all; see Figure 1 for locations of these sampling stations) using a van Veen grab. Sampling station substrates ranged from coarse to more fine sandy sediments. Samples and controls (including extraction, PCR and sequencing blanks) were processed in the Cawthron laboratory following the standard operating procedure (SOP) developed for monitoring salmon farms in the Marlborough Sounds. Samples were sequenced for bacterial eDNA at Auckland Genomics. We estimated diversity, extracted the proportion of bacterial Eco-Groups (EGs)⁶ matching our national GenCodeID⁷ database (440 entries), and calculated b-MBI values for each sample.

⁶ Eco-groups are assigned using quantitative regression spline analyses of ASV-based bacterial taxa that show consistent preferences for a given enrichment stage, and which allow their classification into six ecological categories, from sensitive to resistant.

⁷ Unique genetic barcodes library corresponding to bacterial sequence data of 440 Eco-Groups in New Zealand.

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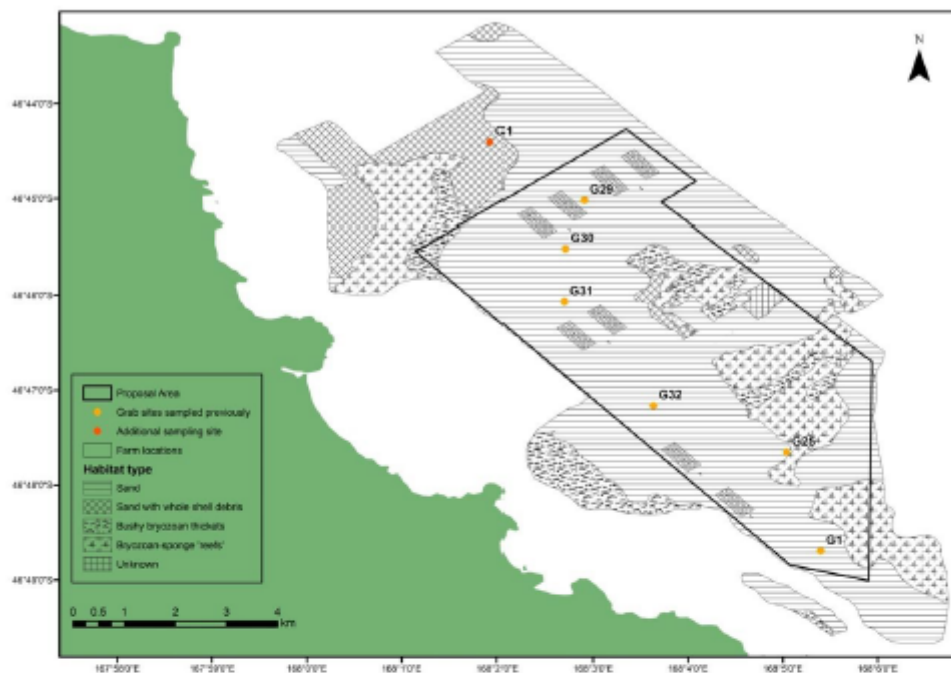


Figure 1. Hananui open ocean aquaculture proposal area (black dashed line) with locations of the seven seabed stations sampled during this survey (C1, G1, G25, G29, G30, and G31).

An additional bacterial dataset obtained in November 2013 from a salmon farm site in Big Glory Bay (BGB) and published in Keeley et al. in 2018¹, was included for comparison. The dataset included triplicate sediment samples from five stations (15 samples total) collected along an organic enrichment gradient: one pen station (0 m), two stations at the inner and outer boundaries of the transitional zone (50 m and 150 m), and two 'control' stations (300 m and 400 m). This low-flow farm was established in 1983 and, at the time of the survey, received feed inputs of approximately 5000 tonnes annually. Comparative analyses of bacterial community composition and structure between the BGB (2013) and Hananui (2021) datasets were visualised using barplots and Principal Component Analysis (PCoA).

Results

We obtained an average of 22,535 (SD = 10,901) high-quality (i.e. post filtration) bacterial 16S sequence reads per sample from the BGB (2013) and Hananui (2021) datasets combined. The number of reads per sample was generally consistent across sites and stations. No contamination was detected in any of the controls, indicating that the laboratory workflow detailed in the SOP was optimal.

The 15 samples collected at BGB in 2013 yielded a total of 5,902 Amplicon Sequence Variants (ASVs), which approximate the taxonomic level of bacterial species. At the family

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level, a clear gradual shift in community composition is observed from highly-enriched sediments at the pen station (0 m) to relatively unenriched sediments at the outer limit of effect stations (300 m and 400 m; Figure 2A, left panel). The enriched samples are dominated by sulphate-reducing bacterial families that occur in anoxic conditions (e.g. Sulfurovaceae, Desulfobacteraceae), while unenriched samples are dominated by families that thrive in more natural, oxygenated sediments (e.g. Vibrionaceae, Rubritaleaceae).

The 21 samples collected at Hananui in 2021 generated a total of 13,253 ASVs or bacterial species. Of these, only 156 ASVs (1.18%) yielded exact matches to ASVs recorded at BGB. Within BGB, the majority of these matching ASVs were found near/at control sites (Table 1).

Table 1. Summary of number and percentage of unique Amplicon Sequence Variants (ASVs, total of 156) shared between Big Glory Bay (BGB) and Hananui, per BGB station (distance in meter to the fish farm pen). Note that total sum in the 'Number' column is 355 because some ASVs are found at more than one station.

BGB Stations	Shared ASVs (BGB/Hananui)	
	Number	%
0 m	10	6.4
50 m	34	21.8
150 m	100	64.1
300 m (Ctrl)	97	62.2
400 m (Ctrl)	114	73.1

At the bacterial phylum level, Hananui sediments are dominated by Proteobacteria (Figure 2A, right panel), which was also observed at the 300 m and 400 m BGB stations. Compared with BGB, Hananui has a greater proportion of Actinobacteriota but only background traces of Bacteroidota and Desulfobacterota. Interestingly, all Hananui stations harbour a unique diversity of Planctomycetota, which are known to play an important role in global carbon and nitrogen cycles, with many species of this phylum capable of anaerobic ammonium oxidation⁸.

Principal Component Analysis (PCoA) showed that bacterial communities at Hananui form a well-separated cluster that is distinct from the bacterial community structure observed across the BGB stations (Figure 2B). Bacterial community richness (measured using the Shannon diversity index) was greater in Hananui sediments compared to BGB. Bacterial assemblages at the BGB farm followed a predictable shift in community cluster, corresponding with the enrichment gradient from pen stations (Figure 2B, bottom) to unenriched stations 300–400 m from the farm (Figure 2B, top).

⁸ Dong X, Lan H, Huang L, Zhang H, Lin X, Weng S, Peng Y, Lin J, Wang JH, Peng J, Yang Y 2022. Metagenomic views of microbial communities in sand sediments associated with coral reefs. *Microbial Ecology*, 3: 1-3.

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To date, a total of 440 distinct bacterial ASVs have been characterised and assigned to specific Eco-Group (EG) categories ranging from very sensitive (EG I) to resistant (EG VI) bioindicators, and are stored in our global GenCodeID database. Out of those 440 ASVs, 230 (~52%) were detected among the BGB samples and 60 (~14%) among Hananui samples (Table 2).

Table 2. Summary of Eco-Group assignments (EG I – VI) for bacteria using the BGB and Hananui data. Eco-Groups are defined as: EG I = very sensitive, EG II = sensitive, EG III = Ubiquitous, EG IV = Transitory, EG V = Opportunistic, and VI = Resistant.

EG	BGB		Hananui	
	Number of ASVs	Percent (%)	Number of ASVs	Percent (%)
I	104	45.2	47	78.3
II	49	21.3	9	15.0
III	2	0.9	0	0
IV	29	12.6	1	1.7
V	35	15.2	0	0
VI	11	4.8	3	5.0

The proportion of bacterial Eco-Groups (EGs) recovered across the enrichment gradient of the BGB farm stations is consistent with previous observations³, whereby sensitive indicators (i.e. EGs I and II) dominate at the control stations and transitory, opportunistic and resistant bacteria (i.e. EGs IV, V, and VI) dominate at the pen and close proximity farm stations (Figure 3A). Hananui sediment samples were dominated by EG I (n = 47; 78.3%), followed by EG II (n = 9; 15%), EG IV (n = 1; 1.7%), and EG VI (n = 3; 5%)⁹. Individual b-MBI values for the Hananui sediment samples ranged between 1.6 and 2.3 (see Figure 3B, Appendix 1). This result corresponds with the unimpacted, natural state end of the enrichment spectrum.

⁹ The total number of ASVs in the GeneCodeID database corresponding to each Eco-Group (EG) are as follows: EG I, 210; EG II, 94; EG III, 6; EG IV, 43; EG V, 38; and EG VI, 49.

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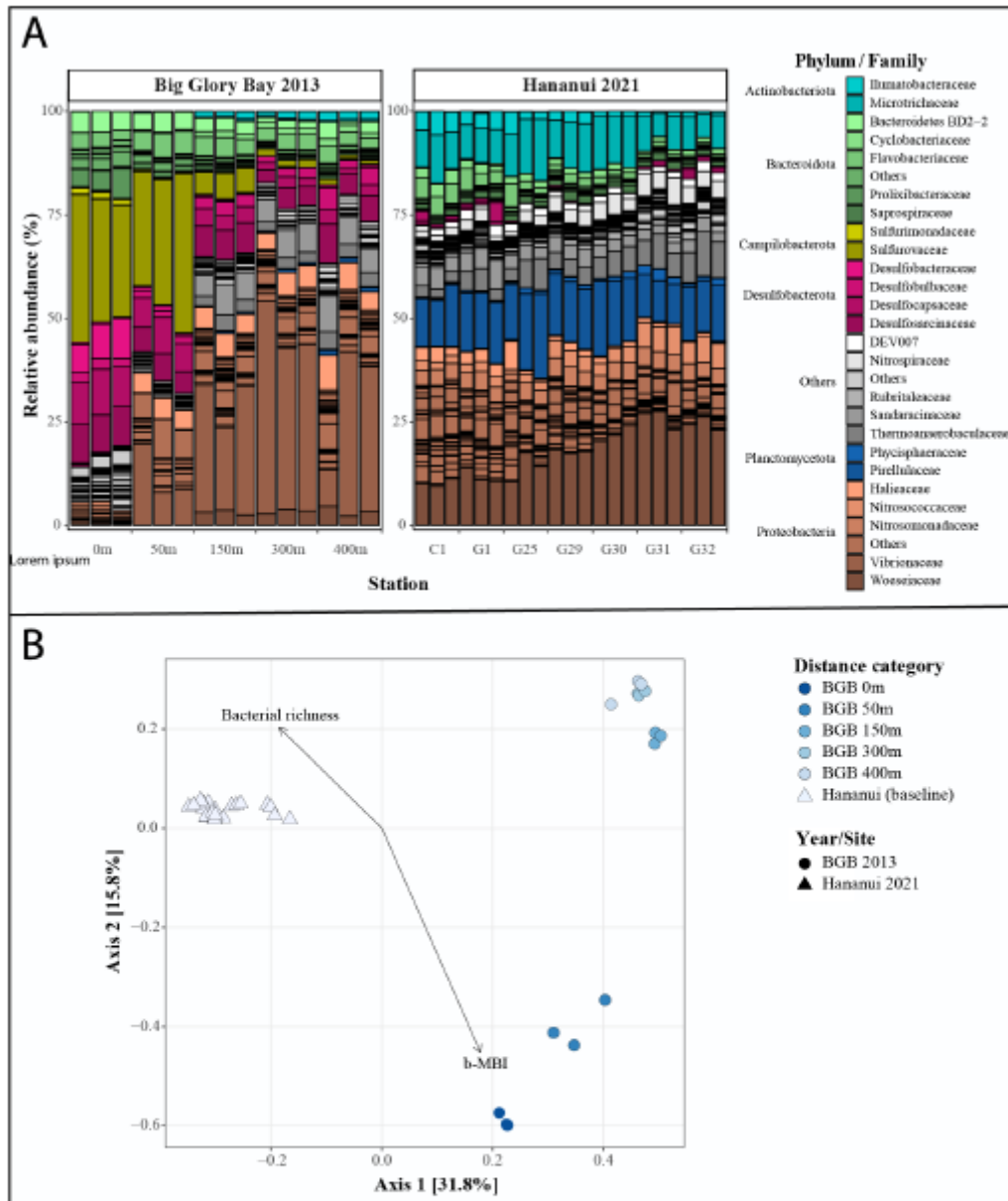


Figure 2. (A) Bacterial community composition at Phylum and Family level for sediments collected in 2013 from 5 stations situated across the enrichment gradient of a salmon farm in Big Glory Bay (BGB, left panel) and for sediments collected in 2021 from 7 stations across the proposed Hananui open ocean aquaculture site (right panel). (B) Two-dimensional Principal Component Analysis (PCoA) plot based on square-root transformed environmental DNA relative read abundance data for bacterial assemblages at all investigated stations from the BGB (2013, circles) and Hananui (2021, triangles) sites. Bacterial richness (Shannon diversity) and bacterial-Metabarcoding Biotic Index (b-MBI) estimates were overlaid as vectors to the PCoA plot. Three replicate samples were collected at each sampling station.

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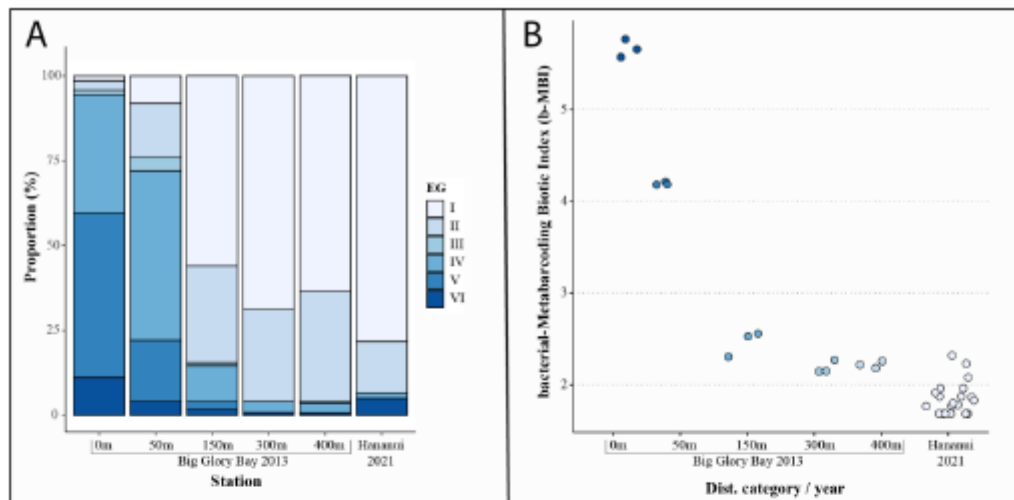


Figure 3. (A) Proportion of bacterial Eco-Groups (EGs) recovered across the enrichment gradient of the Big Glory Bay (BGB) farm stations and from the combined Hananui stations. Eco-Groups are defined as: EG I = very sensitive, EG II = sensitive, EG III = ubiquitous, EG IV = transitory, EG V = opportunistic, and VI = resistant. (B) Bacterial Metabarcoding Biotic Index (b-MBI) values obtained for each investigated BGB (n = 15) and Hananui (n = 21) sample.

Discussion

Seabed impacts resulting from fish farm activities in the Marlborough Sounds are currently determined by measuring chemical properties of sediment and changes in macro-infaunal diversity. These parameters are incorporated into the ES index¹⁰, which provides regulators and producers with a measure of environmental impact at a specific site. The recent validation of our eDNA-derived (b-MBI) method³ for this region represents a promising new technique for determining the level of benthic enrichment. This is likely to be of particular use at the Hananui site, where infaunal communities are scarce, and traditional metrics may be difficult to calculate¹¹.

This is the first eDNA-based survey and b-MBI assessment at the prospective Hananui open ocean aquaculture site. Using our established sample processing and analytical workflows², we obtained high-quality sequencing results indicating that our SOP is also optimal for use at exposed marine sites. These preliminary data revealed that abundant and diverse bacterial assemblages are present across the Hananui site, including the six most common bacterial phyla encountered in natural marine sediments. Bacterial species richness was high at the Hananui site, contrasting with macrofaunal assemblages which are known to be relatively scarce at this location. Interestingly, unlike macrofauna and derived diversity indices obtained from four stations (Appendix 1), bacterial community composition was relatively

¹⁰ Keeley N, Forrest BM, Macleod C 2013. Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management. *Marine Pollution Bulletin* 66: 105-116.

¹¹ Bennett H, Smeaton M, McGrath E, Newcombe E 2020. Assessment of seabed effects associated with farming salmon offshore of northern Stewart Island / Rakiura. Prepared for Ngāi Tahu Seafood Resources. Cawthron Report No. 3315A. 93 p. plus appendices.

consistent across replicate samples and stations, including those comprised of varying substrate types (i.e. C1, located in coarse sediment).

A number of valuable observations were gathered from the comparative analysis of the BGB (2013) and Hananui (2021) stations. First, we showed that phylum level bacterial diversity at Hananui was most similar to BGB 'control' stations (300 m and 400 m), though Hananui communities also contained exclusive phyla, presumably related to the differing environmental conditions. Second, there was minimal overlap between sites at the bacterial species level, with only 1.18% of Hananui ASVs occurring across BGB stations. This translated into clearly distinct bacterial community structures between Hananui and BGB sites, as visualised in the PCoA plot (Figure 2B). This result is not surprising given fundamental differences in environmental conditions between the two sites in terms of exposure (high- versus low-flow), sediment type (sand versus mud), and organic matter accumulation (low versus high). Notably, most, if not all of the samples from BGB in 2013 were from seabed that is likely to be enriched to some degree, as the bay has had prolonged exposure to fish farming and is naturally depositional (due to weak currents). Therefore it is likely to be moderately naturally enriched relative to the exposed coastline in the area of the Hananui site. However, when considering that the BGB data were collected nine years ago, we cannot exclude the possibility that the lack of similarity between sites is also due to temporal factors. Additional sampling from both BGB and Hananui stations is required to verify this hypothesis.

Another aim of this preliminary study was to compare the baseline bacterial data from the Hananui site against our GenCodeID database to identify the number and class types of ASVs assigned to EGs and to calculate the b-MBI value for each station. We recovered a relatively low, but appreciable, number (60 out of 440) of ASVs present in the database, and these were largely dominated by EGs I and II, which represent the 'pristine' end of the ES spectrum¹². Thirty-seven of these ASVs were also detected from BGB stations (35 [94.6%] of which were found at the BGB control stations), with the remaining 23 ASVs matched ASVs from the Marlborough Sounds. The calculation of b-MBI confirmed that all Hananui stations are currently under pristine/natural conditions, with b-MBI values < 2.5, which corresponds to values typically found at unimpacted control sites. It is worth noting that most ASVs currently found in the GenCodeID database were characterised from salmon farms in the Marlborough Sounds, but with a known proportion (~50%) being universally distributed in fish farms environments as far as Norway¹³. We have observed that organically impacted sediments tend to converge on similar bacterial EGs, likely due to similar ecological processes (benthic function) as well as similar inoculation pressure of bacterial strains originating from salmon faeces or feed inputs.

We predict that once aquaculture operations start at Hananui, there will be a gradual replacement of naturally occurring bacterial taxa with those thriving in organically enriched

¹² Due to the lack of enrichment at the Hananui site, low representation of EGs III – VI was expected.

¹³ Keeley N, Laroche O, Birch M and Pochon X (2021) A Substrate-Independent Benthic Sampler (SIBS) for Hard and Mixed-Bottom Marine Habitats: A Proof-of-Concept Study. *Frontiers in Marine Science* 8:627687.

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sediments, which will increase both the number of known bacterial EGs and the precision of b-MBI index at this site.

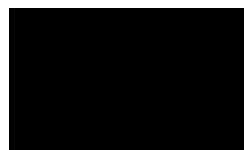
Recommendations

Our conclusion is that the b-MBI approach is a promising tool for future monitoring of the Hananui open ocean aquaculture site. We recommend that additional sampling and comparative analyses between the BGB and Hananui sites be conducted to (1) better understand the temporal and seasonal factors that may affect the distribution of bacterial indicators from both sheltered and exposed sites around Stewart Island, (2) identify additional bacterial Eco-Groups from BGB farms to consolidate our GenCodeID database for future applications in the region, and (3) assessment of how b-MBI reflects changes in traditional measures of enrichment (i.e. macrofauna indices, organic matter, redox, and sulphides).

We trust that the above assessment provides an informative update. However, please do not hesitate to contact us if you require further information.

Yours sincerely,

Scientists



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Molecular Ecologist
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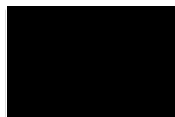


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Appendix 1. Descriptors, sediment chemical properties, macrofauna variables, and calculated indices for sampling stations at the proposed Hananui open ocean salmon farm during the October 2021 survey. NC = not calculated.

Station	Rep	Lat	Long	Depth (m)	Redox (EhNHE, mV)	pH	UV sulphide (µm)	Abundance (N)	No. taxa (S)	Evenness (J')	Richness (d)	SWDI	AMBI	BQI	b-MBI
C1	A	-46.740	168.032	34	426	6.42	NA	200	41	0.8	7.55	2.97	2.45	7.22	1.78
C1	B	-46.740	168.032	34	402	7.36	289.26	173	39	0.6	7.37	2.18	1.85	8.21	1.77
C1	C	-46.740	168.032	34	361	7.26	220.05	205	33	0.72	6.01	2.51	2.26	6.61	1.80
G1	A	-46.812	168.090	35	410	7.38	46.14	NC	NC	NC	NC	NC	NC	NC	1.88
G1	B	-46.812	168.090	35	407	7.92	69.21	NC	NC	NC	NC	NC	NC	NC	1.69
G1	C	-46.812	168.090	35	418	8.17	51.46	NC	NC	NC	NC	NC	NC	NC	1.77
G25	A	-46.794	168.084	35	379	7.41	95.83	8	4	0.77	1.44	1.07	2.00	1.45	1.69
G25	B	-46.794	168.084	35	410	7.93	83.41	29	7	0.78	1.78	1.52	4.07	3.70	1.69
G25	C	-46.794	168.084	35	406	7.61	161.49	6	3	0.92	1.12	1.01	2.50	1.48	1.88
G29	A	-46.750	168.049	37	399	8.12	154.39	NC	NC	NC	NC	NC	NC	NC	1.87
G29	B	-46.750	168.049	37	400	7.98	37	NC	NC	NC	NC	NC	NC	NC	1.83
G29	C	-46.750	168.049	37	383	8.17	81.63	NC	NC	NC	NC	NC	NC	NC	2.32
G30	A	-46.759	168.045	35	402	7.99	47.91	31	20	0.95	5.53	2.85	1.96	5.95	2.23
G30	B	-46.759	168.045	35	417	7.81	47.91	27	14	0.95	3.94	2.52	2.28	4.84	1.69
G30	C	-46.759	168.045	35	420	8.05	70.98	3	3	1	1.82	1.10	1.50	1.28	1.69
G31	A	-46.768	168.045	31	424	8.14	49.69	NC	NC	NC	NC	NC	NC	NC	1.69
G31	B	-46.768	168.045	31	420	8.17	56.79	NC	NC	NC	NC	NC	NC	NC	1.96
G31	C	-46.768	168.045	31	429	8.22	44.37	NC	NC	NC	NC	NC	NC	NC	1.96
G32	A	-46.786	168.061	26	469	7.75	37.00	22	12	0.93	3.56	2.31	1.74	4.74	1.92
G32	B	-46.786	168.061	26	429	8.02	NA	24	12	0.93	3.46	2.30	1.67	6.38	1.69
G32	C	-46.786	168.061	26	396	7.67	88.73	6	5	0.97	2.23	1.56	1.50	2.33	2.08

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